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SEX CHROMATIN PATTERN IN SEMINIFEROUS TUBULE DYSGENESIS AND OTHER TESTICULAR DISORDERS: RELATIONSHIP TO TRUE HERMAPHRODISM AND TO KLINEFELTER'S SYNDROME,

WITH A REVIEW OF GONADAL ONTOGENESIS*†

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THE discovery by Barr and his associates of a sex difference in the interphase nuclei of somatic cells in certain mammals (2–6), including primates, has provided a long-needed practical method for assessing the sex chromosome constitution. Since this technique was first applied in man in 1953 (7), modifications (8–11) and several new developments have been reported (12–17).

In the investigation of anomalies of sex development, clinical application of Barr's method has produced important results (7, 18–23). Apart from its ancillary rôle in diagnosis, cytologic sexing, or the determination of sex by chromatin pattern, has contributed significantly to our understanding of the disordered development in these afflictions (23, 24).

Recently, chromosomal sex has been studied in testicular disorders. Nelson (25), Plunkett and Barr (26, 27), and Bradbury, Bunge and Bocca-

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bella (28) were the first to describe a female chromatin pattern in apparent males with Klinefelter's syndrome. Subsequently, this finding has been reported by the authors and Barr (1), and by others (29–31).

In the present investigation, chromosomal sex was determined in a group of 65 males with a variety of testicular disorders. Among these, a female chromatin pattern was detected in 9 patients, all of whom presented with a history of sterility or were found to have atrophic testes or signs of Klinefelter's syndrome. The histopathology of the testes varied from germinal aplasia to tubular fibrosis. The sex chromatin pattern and the associated testicular pathology indicated a gonadal disorder of a basic congenital nature principally evident in the seminiferous tubules. Accordingly, we propose the term *"seminiferous tubule dysgenesis"* for this condition. Of the chromosomal males with tubular fibrosis or germinal aplasia, 23 patients were found who had testicular lesions and clinical findings similar to those observed in the 9 chromosomal females. These chromosomal males have also been included under the diagnosis of seminiferous tubule dysgenesis. Apparent males with seminiferous tubule dysgenesis and a female chromatin pattern appear to represent the first example of a complete sex reversal in the human.

We introduce the name seminiferous tubule dysgenesis with due consideration, into a field already encumbered by a complicated and diverse terminology. As originally described, cases of Klinefelter's syndrome had azoospermia, gynecomastia, a variable degree of eunuchoidism, elevated urinary gonadotropins, and atrophic testes with atrophy and hyalinization of the seminiferous tubules in which Leydig cells were preserved (32). Heller and Nelson (33) and Howard *et al.* (34) later showed that gynecomastia as well as eunuchoidism was a variable feature of this syndrome. In our group of patients, severe atrophy of the testes, although usual, was not constant; nor was an elevated level of urinary gonadotropins uniformly found on single determinations. Hypospadias and cryptorchidism are present in some cases (27, 103). Furthermore, in certain cases the testicular lesion differs in some respects from that originally described. In all, "Klinefelter's syndrome" in its present usage is difficult to define. There is a need for improved terminology. The generic title "seminiferous tubule dysgenesis" describes a group of testicular disorders of congenital origin. Some, but not all, of these patients, irrespective of their chromosomal sex, have the clinical features described by Klinefelter, Reifenstein and Albright (32). Moveover, not all cases classified as Klinefelter's syndrome can be included under the diagnosis of seminiferous tubule dysgenesis.

SUBJECTS, METHODS AND RESULTS

The sex chromatin pattern was determined in 65 patients with testicular disorders who presented with either sterility, eunuchoidism, cryptor-

TABLE 1. SALIENT FINDINGS IN 32 CASES OF SEMINIFEROUS TUBULE DYSGENESIS

Sex chromatin pattern	*Tubular fibrosis*		*Germinal aplasia*	
	Female*	Male	Female	Male
Number of patients	8	8	1	15
Age (yrs.)	15–32	27–47	25	22–36
Male secondary sexual development				
Normal	4	6	1	15
Eunuchoid (moderate to severe)	4	2	0	0
Gynecomastia	5	3	0	0
Severe atrophy of testes	5	4	0	3
Azoospermia	8	8	1	15
Elevated FSH (>80 m.u.)	4/6	3/3	1†	0/0
Familial cases	0	3	1	0

* Includes 1 patient with myotonia dystrophica.
† Of three determinations.

chidism, atrophic testes, or signs of Klinefelter's syndrome. Testicular biopsy specimens were obtained from all but 1 patient. In Tables 1 and 2 are the results of cytologic sex determination and the findings on testicular biopsy in each subject. A female chromatin pattern was found in 7 patients with tubular fibrosis and absent or rare germ cells, and in 1 patient with

TABLE 2. SEX CHROMATIN PATTERN IN OTHER TESTICULAR DISORDERS

Testicular disorder	Sex chromatin pattern	
	Male	Female
Tubular fibrosis or germinal aplasia, secondary or of unknown etiology (including cases with cryptorchidism)	24	0
Partial germinal aplasia	1	0
Spermatogenic arrest		
Complete	5	0
Incomplete	1	0
Retarded maturation of seminiferous tubules	1	0
Congenital anorchia with microgenitalia	1	0
Total	*33*	*0*

germinal aplasia. In addition, a female chromatin pattern was detected in a 15-year-old boy with all the clinical features of Klinefelter's syndrome and a titer of urinary gonadotropins in excess of 80 mouse units per day, who did not have a testicular biopsy. The pathologic (see subsequent section) and clinical findings in these 9 patients with a female chromatin pattern formed the basis for the diagnosis of seminiferous tubule dysgenesis. This diagnosis was accordingly made in 8 chromosomal males with tubular fibrosis and in 15 with germinal aplasia,[1] among patients with superficially similar testicular lesions.

In Table 1 the salient findings are summarized for the entire group of 32 patients with seminiferous tubule dysgenesis according to the type of testicular pathologic lesion involved. In all these patients, male secondary sexual characteristics developed to some degree, ranging from normal to eunuchoid. No consistent clinical or pathologic feature served to differentiate seminiferous tubule dysgenesis in the chromosomal males from that in the chromosomal females. In 1 patient with female nuclei, the tubular fibrosis was associated with myotonia dystrophica. The familial aspect of this condition is noteworthy. The patient with germinal aplasia and a female chromatin pattern was found to have an older sibling with tubular fibrosis and a male chromatin pattern. An additional male sibling was reported to be normally developed and the father of one son and two daughters. Two of the patients with tubular fibrosis and male nuclei were brothers.

Chromosomal males with tubular fibrosis or germinal aplasia were excluded from the group with seminiferous tubule dysgenesis when the testicular pathologic picture (see subsequent section) differed significantly from that found in the chromosomal females, although some of these patients had the clinical features of Klinefelter's syndrome. Included in this category were chromosomal males with spermatogenic activity in more than occasional tubules and cases in which agents known to cause testicular degeneration could be implicated (36). However, some of the patients in this group may well have had congenital testicular defects.

In Table 2 are listed the results of cytologic sexing in other testicular disorders which have been studied. The diagnosis in these cases is based upon the testicular pathology (37, 38).

The sex chromatin pattern was determined in various tissues, including testes, oral mucosa, skin and breast. Bilateral testicular biopsies were performed in patients with seminiferous tubule dysgenesis, as well as in most other patients. Testicular specimens were fixed in Bouin's fluid or methyl alcohol, embedded in paraffin, sectioned at 5 microns, and stained with hematoxylin and eosin and Masson's stain. Methyl alcohol fixation proved adequate for Feulgen staining, but specimens fixed only in Bouin's

[1] Dr. A. R. Sohval kindly made available for study the specimens from 2 cases (35).

fluid yielded poor results with this stain. Testicular sections in which good Feulgen stains were obtained confirmed the results of cytologic sexing obtained by the hematoxylin and eosin technique. Biopsy specimens of skin were treated according to the method of Moore, Graham and Barr (7, 19). The methods of Moore and Barr (8) and of Marberger, Boccabella and Nelson (9) were used for the preparation of smears obtained from the oral mucosa. In replicate oral mucosal smears fixed and stained by these two methods, the results were similar. Accordingly, the more rapid method of Moore and Barr, utilizing Papanicolaou fixative and cresyl echt violet stain, was preferred, and the Feulgen procedure was employed largely as a confirmatory test when a female chromatin pattern was observed. More than one tissue was used for cytologic determination of sex in all but 1 of the 9 chromosomal females;[2] in 8, this included an oral mucosal smear.

Cytologic sexing was performed independently on all specimens by two of the authors. In each specimen 200 or more cells were examined for sex chromatin according to the method described by Barr (oral mucosal smears obtained in quadruplicate were regarded as a single specimen). Only well preserved nuclei in oral mucosal smears and biopsy specimens were selected for counting. The diagnosis of a female chromatin pattern was based upon detection of the sex chromatin mass in 40 per cent or more of the nuclei. In the patients with male-type nuclei, less than 7 per cent of the nuclei contained a chromatin mass comparable to that seen in females. This sharply-defined sex difference is similar to the results obtained in this laboratory with specimens of good technical quality from normal individuals.

The nuclei of Leydig cells in properly fixed and sectioned testicular biopsy material, stained with hematoxylin and eosin, were found to be eminently satisfactory for sexing (Fig. 1 and 2). This permitted supplementation of data by the review of a large number of testicular biopsies previously performed. Sertoli cells generally were not as suitable for sex determination and tended to show a lesser percentage of sex chromatin than the Leydig cells.

No discrepancy has been observed between the sex chromatin content of nuclei in the various tissues from the same individual when technical details were scrupulously followed and only nuclei satisfactory for sexing selected. This has been true in the normal subject as well as in a considerable number of cases with disturbances in sex differentiation.[3]

Testicular Pathologic Changes in Seminiferous Tubule Dysgenesis

Table 3 summarizes the pertinent histologic and cytologic data. The changes observed in the seminiferous tubules were qualitatively consistent in all patients. There were quantitative variations from case to case and from one area to another in the microscopic sections from the biopsy speci-

[2] In addition, Barr has confirmed the cytologic diagnosis in 5 of these cases, including the patient with myotonia dystrophica.

[3] Plunkett and Barr (27) found fewer female-type neutrophils than usually occur in normal females on studying blood smears by the method of Davidson and Smith (11) from 2 cases of seminiferous tubule dysgenesis in which female nuclei were observed in other tissues examined. The significance of this discrepancy, although potentially of great interest, cannot be assessed until further data are available.

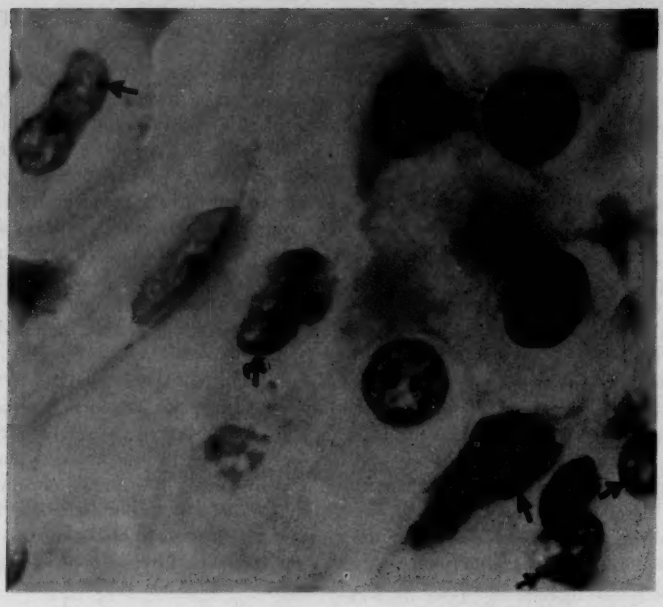


FIG. 1. Seminiferous tubule dysgenesis with tubular fibrosis and female chromatin pattern. Tubule with minimal thickening of the tunica propria. Typical peripheral sex chromatin masses are present both in the Sertoli cells (upper left) and in the adjacent Leydig cells (lower right). Hematoxylin-eosin, 1863×.

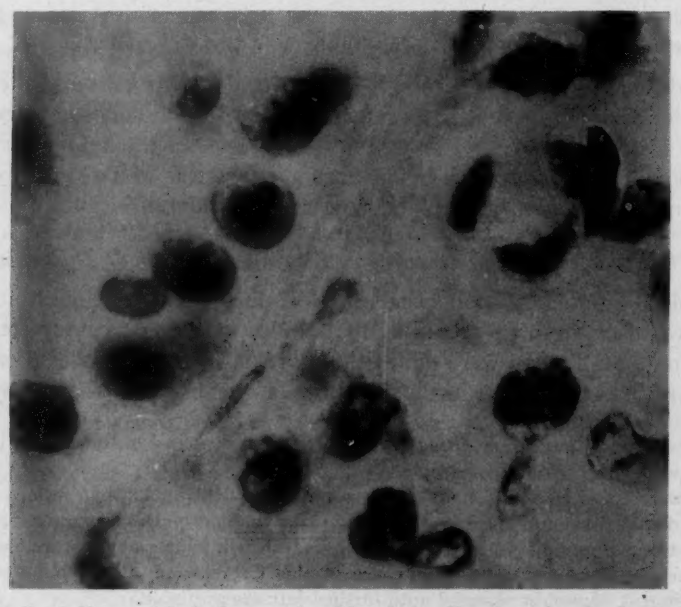


FIG. 2. Seminiferous tubule dysgenesis with tubular fibrosis and male chromatin pattern. Tubule without thickening of the tunica propria. No sex chromatin is seen. Hematoxylin-eosin, 1863×.

TABLE 3. HISTOPATHOLOGY OF SEMINIFEROUS TUBULE DYSGENESIS*

Patient	Age (yrs.)	Chromosomal sex	Seminiferous tubules				Leydig cells						Percentage of nuclei with sex chromatin		
			Tubular fibrosis	Germ cells	Dysgenetic tubules	Degeneration of Sertoli cells	Number	Secretory activity	Crystalloids	Pigment	Spindle cells	Degenerative changes	Leydig cells	Vessels	Sertoli cells
W.S.	27	F	3	1	3	3	3	3	1	1	2	1	63	62	45
S.O.	15	F	3	1	3	3	2	2	1	2	2	1	58	58	15
L.H.	16	F	3	0	3	3	3	3	1	1	2	1	66	—	37
H.S.	23	F	3	0	3	3	2	2	1	1	2	2	52	—	7
I.H.	23	F	3	0	3	3	3	3	1	1	2	1	67	76	35
A.S.†	32	F	3	0	3	3	3	3	1	2	2	1	70	55	75
P.M.	29	F	3	0	3	3	3	3	1	2	3	1	40	45	9
A.A.§‡	24	F	1	0	1	2	2	3	1	2	1	1	48	—	24
F.M.¶	47	M	3	0	3	3	2	2	1	1	2	1	6% or less		
J.M.¶	36	M	2	0	3	2	2	1	2	1	2	2			
H.W.	30	M	2	0	1	2	2	1	1	1	3	2			
M.R.	32	M	2	0	1	2	3	2	1	1	3	2			
H.B.	34	M	2	2	2	1	3	2	1	2	2	1			
H.C.	36	M	2	1	2	2	2	2	1	2	3	2			
C.C.	42	M	3	0	1	3	1	3	1	1	1	1			
B.A.‡	27	M	2	1	1	3	1	3	1	1	3	3			

* The presence of occasional germ cells is indicated by 1 and that of spermatogenesis by 2. All other features are graded from 1 to 3 to designate increasing number, amount or severity of lesions.

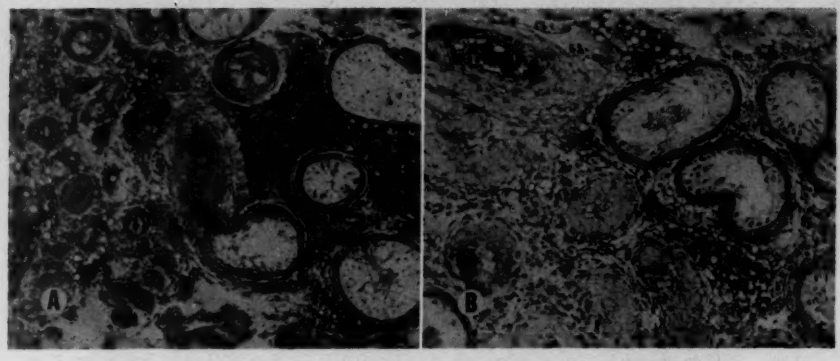
† Associated with myotonia dystrophica.

‡ and ¶ Sibling pairs.

§ Case of germinal aplasia. In the 15 genetic males with germinal aplasia, the histopathologic findings were identical.

men of a single patient and, sometimes, between the left and right testis. It was only after the cases of tubular fibrosis had been classified according to their chromosomal sex that certain differences emerged between the male and the female groups. No single feature could be identified which was characteristic for one group.

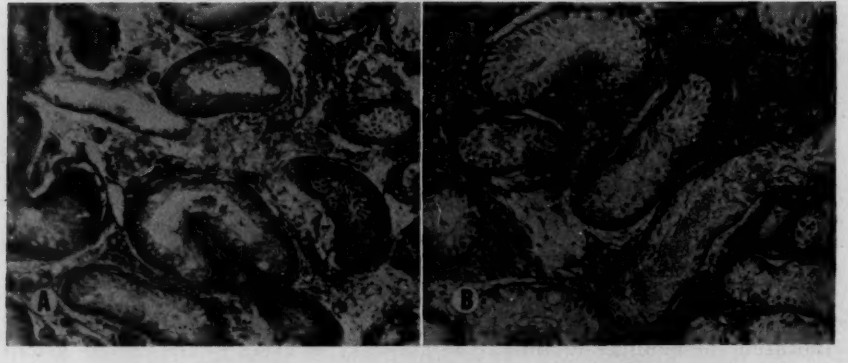
In general, the cases with female nuclei showed only rare or absent germ cells, nonhomogeneous tubular fibrosis with small sclerotic immature tubules, preserved immature tubules, occasional active or fibrosed mature tubules, and marked clumping of interstitial cells (Fig. 3a). The patients with male chromatin pattern displayed all degrees of the same changes (Fig. 3b, 4a); these alterations in some cases culminated in lesions which imitated the changes found in the majority of cases of the female group (Fig. 3b). Differences in the intensity of the process are evident from other reports (34, 36, 39–41) and are as marked as variations in the clinical picture. The authors recognize with Nelson (39) and Sniffen *et al.* (36, 40) that the testis reacts as a unit and that, at least at the time of biopsy, Leydig cells, tubular wall, Sertoli cells and germ cells are all altered. Thus, the



A B

FIG. 3. **A.** Seminiferous tubule dysgenesis with tubular fibrosis and a female chromatin pattern (H.S.). The tubules contain vacuolated Sertoli cells. The tunica propria is thickened. Some tubules appear as small collagenous cords. Clumping of Leydig cells is evident. Masson's trichrome, 126×. **B.** Seminiferous tubule dysgenesis with tubular fibrosis and a male chromatin pattern (H.C.). Note the similar appearance and the presence of small dysgenetic tubules. Masson's trichrome, 126×.

term seminiferous tubule dysgenesis summarily defines one feature which is considered basic from both a pathogenic and morphologic point of view. Furthermore, it is realized that no definite statement about the absence of spermatogenesis or of immature tubules can be drawn from a single sample. Serial sections at multiple levels were examined, but serial sections of the



A B

FIG. 4. **A.** Seminiferous tubule dysgenesis with tubular fibrosis and a male chromatin pattern (B.A.). In this field, the sclerosis is less advanced than in Figure 3. Masson's trichrome, 126×. **B.** Seminiferous tubule dysgenesis with germinal aplasia and a female chromatin pattern in the sibling (A.A.). The tubules show minimal thickening of the tunica propria. Masson's trichrome, 126×.

whole biopsy specimen were not made to determine whether the changes observed were identical along a given tubule.

Seminiferous tubules and germ cells

Considerable thickening of the tubular wall was seen in all cases, except in the sections from the patient with germinal aplasia and a female chromatin pattern (Fig. 4b). The germinal epithelium was absent from most tubules of two specimens in which primary spermatocytes were seen. The sclerosis could be attributed to thickening of the tunica propria by the deposition of homogeneous or laminated collagenous substance. The basement membrane was intact in the moderately fibrosed tubules, but appeared to be incorporated in the hyalinized tunica propria of the tubules with advanced fibrosis. Two types of seminiferous tubules could be recognized, each presenting a distinctive pattern of regressive changes.

Large tubules of a diameter equivalent to that of a normal mature tubule had a variegated appearance. When there was only minimal thickening of the tunica propria one could occasionally see foci of disorganization of spermatogenesis, sloughing and spermatogenic arrest. This was found in only 2 patients, both of whom had male nuclei. Fibrotic changes were accompanied by involution of germ cells in the few tubules in which germ cells were present. When fibrosis was advanced, the lumen was lined solely with Sertoli cells. There was a progressive decrease in the diameter of the tubules, which eventually became collagenous strands; in the latter the contours of the infolded tunica propria were sometimes still recognizable. The involution could be followed step by step in most specimens. Some of the tubules with little or no fibrosis were likewise lined only with Sertoli cells and had the appearance described in germinal aplasia. Complete absence of elastic fibers, or the presence of only a small number of thin elastic fibers at the periphery of the thickened tunica propria, was noted by Sniffen *et al.* (36) and by de la Balze *et al.* (42). Besides the small diameter of the tubules and their insufficient coiling, this is a useful criterion of prepuberal degenerative changes, since elastic fibers normally appear first at puberty and either persist or increase in number in acquired tubular fibrosis and with age. The observation of lesions of increasing severity in different tubules as evidence of progressive sclerosis seems justified at least for a part of the tubules.

There appeared to be a hiatus in the spectrum of progressive lesions between the type of involutive changes seen in larger tubules and the different pattern presented by other tubules. In these small tubules the thickening of the tunica propria was sometimes minimal in spite of their reduced diameter. The lumen was narrow or absent. The cells lining the tubules had the appearance of prepuberal undifferentiated cells (Fig. 5). The cross-sections were frequently widely separated from each other, suggesting prepuberal growth arrest with insufficient coiling of the tubules. Normal testes may contain a few of these abortive elements. A significant number of similar structures have been observed in some cryptorchid testes (43), and we have seen them in the gonads of hermaphrodites. When these immature tubules had undergone fibrosis, there was often hyalinosis of the cells themselves in addition to peritubular fibrosis. In contrast to the deeply stained collagenous tissue surrounding the larger tubules, the sclerotic immature tubules were frequently lightly stained. The foregoing findings and the very small diameter of these structures suggested that the fibrosis occurred in tubules which were in an early stage of maturation. It also implied that several of the tubules were *dysgenetic*, in that they were

immature at or after puberty, despite scrotal position of the testis and despite signs of secondary sexual development. These ducts had atrophied in a somewhat peculiar manner and this could account for the large number of narrow collagenous cords seen in the testes with this type of tubular fibrosis (Figs. 3, 4). The proportion of small dysgenetic tubules to large, mature tubules was different in each case.

The changes described in both larger and smaller tubules were found to some extent in all patients with seminiferous tubule dysgenesis associated

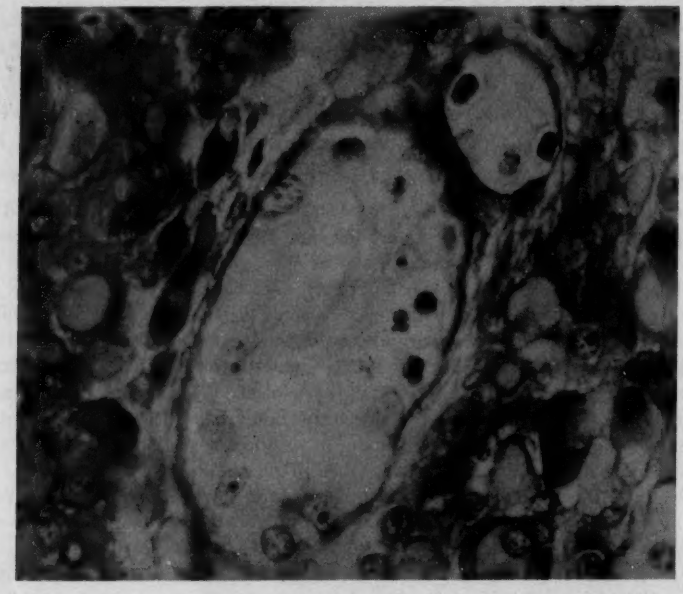


FIG. 5. Tubule with occasional mitoses of germ cells in a case of seminiferous tubule dysgenesis with female chromatin pattern. Note the hypoplastic tubule above and to the right of the larger tubule. Masson's trichrome, 693×.

with tubular fibrosis regardless of the sex chromatin pattern. In the group of chromosomal males the appearance of the tubules was usually more homogeneous, both in degree of maturation and in extent of fibrosis; also, dysgenetic tubules were less common. In the patient with germinal aplasia and female nuclei, a hiatus was observed, in that only scattered very small fibrotic tubules were seen amidst regular large tubules, and no intermediate stages were present. In the patients with tubular fibrosis and a female chromatin pattern one could find both immature tubules with and without sclerosis. In one of them, a case of myotonia dystrophica, most tubules were of the immature type and only a few sclerotic large tubules were seen.

The Sertoli cells usually were well preserved as long as thickening of the

tunica propria was minimal. In the tubules with advanced fibrosis, degenerative changes were apparent, including nuclear pyknosis and karyolysis, and formation of large vacuoles and cytoplasmic smudges. In general, there was good correlation between the degenerative changes, the thickness of the tubular wall and the decrease in diameter of the tubule. Difficulty encountered in "sexing" the nuclei of Sertoli cells might be due to the described alterations. In some tubules the Sertoli cells were smaller, with round finely stippled nuclei and granular pigmented cytoplasm which resulted in an appearance closely simulating Leydig cells.

Leydig cells

Changes in the distribution or cytologic appearance of Leydig cells were constant. Marked clumping or a pseudo-adenomatous pattern was found in 6 out of 8 cases with female nuclei and in 2 out of 8 cases with male nuclei. The apparent increase in number of Leydig cells in severely atrophic testes is probably only relative, and in some cases it is likely that there is an actual deficiency.

There was no consistent correlation between clinical signs of secondary sexual development and the degree of apparent hyperplasia of the Leydig cells or the cytologic signs of active secretion. Usually, however, features considered typical for normal secretory activity (36, 39, 41) were noted in the majority of the Leydig cells found in large clumps. Clumping and active secretion were more common in the group with female nuclei. The amount of pigment and crystalloids was about equal in the active cells of both groups.

The interstitial cells designated as functional had a distinct nucleus, large polygonal or rounded cytoplasm containing clear peripheral vacuoles, clumps of fine eosinophilic granules and occasionally crystalloids and pigment granules. These cells probably represent the mature stage of the life cycle of Leydig cells (44). It is not known where in the cycle the fusiform cells seen in the stroma of the glands should be placed. These spindle-shaped elements had denser nuclei than the normal Leydig cell and a darker cytoplasm. They were larger than fibroblasts and did not have the latter's fusiform nuclei. They were clearly separated from the clumps of normal interstitial cells, and only occasional cells were observed which could be considered as representing an intermediate stage. Whether they represented an unusual type of involution by regression (though they did not contain the large amount of pigment seen in their counterpart in the normal testis) or an immature phase of development, is unsettled. These cells were somewhat more commonly observed in the patients with male nuclei. In this same group, degenerative changes were also more prominent than in patients with female nuclei, and showed some correlation with an absence of marked hyperplasia of the interstitial cells. Degenerative changes in Leydig cells were seen in all cases; even when minimal, they were always more extensive than in a normal testis. Contrary to the observation of de la Balze *et al.* (41), no consistent topographic relationship was found between well-preserved tubules and active Leydig cells.

Stroma

The occasional areas of interstitial edema seen in the testis of both groups appeared to be an artefact in some biopsy specimens. In others, the edema seemed to follow the focal atrophy of interstitial cells which left a loose connective tissue with rare scattered spindle-shaped cells.

Vessels

Thickening of the media of small arteries and arterioles was a frequent finding. It was considered a consequence of testicular atrophy. A similar phenomenon is commonly observed in other atrophic organs.

Counts of sex chromatin

In all the cases with female-type nuclei, 40 per cent or more of the Leydig cells contained a typical chromatin mass in juxtaposition with the nuclear membrane. Similar counts were obtained in the nuclei of young fibrocytes, of smooth muscle fibers of the vessels, and of epithelial cells of the oral mucosa and skin. However, the sex chromatin was not found with the same regularity in Sertoli cells of the cytologic females, in which the frequency of nuclei with sex chromatin varied between 7 and 75 per cent. The chromatin masses were typical in staining properties, location, shape and size. Whether the discrepancy observed in some patients with female nuclei between the incidence of sex chromatin in Sertoli cells and that in other cells is of significance is unknown. The problem is unlikely to be resolved until "healthy" Sertoli cells are observed for comparison in chromosomal females.

Significance of Testicular Histopathologic Findings

Atrophy, absence of germ cells and tubular fibrosis are nonspecific changes and may be observed with aging and in testicular disorders of known exogenous origin. Immature hypoplastic seminiferous tubules are seen in the testes of some patients with tubular fibrosis, germinal aplasia. cryptorchidism (43) and hermaphrodism. When preserved, these small tubules are lined with undifferentiated cells. When fibrosed, their small diameter sets them apart from tubules which undergo fibrosis after having reached adult size. They are considered dysgenetic because they do not develop normally and do not show puberal changes. Their occurrence in seminiferous tubule dysgenesis (particularly in the familial cases and in the patient with myotonia dystrophica—a heritable disease) but not in purely extrinsic testicular lesions, supports the contention that these tubules are congenitally defective. Moreover, the fact that such tubules were found in the chromosomal females with seminiferous tubule dysgenesis suggests that they are a stigma of defective gonadogenesis (*see* Discussion).

SELECTED CASE REPORTS

Seminiferous tubule dysgenesis with Klinefelter's syndrome in a chromosomal female with myotonia dystrophica.

A.S. (P.H.#03-27-12), a 32-year-old male, first noted enlargement of both breasts at the age of 17 years; otherwise puberty and adolescence were uneventful. Although the patient was in good health and served in the Army for six years during and following World War II, he always thought his testes were small in size. His marriage at age 22 was childless and ended in divorce after four years. During the four years before admission, clumsiness and impaired ability to relax his hands after gripping developed, and became progressively worse. His speech became thick. Anorexia, generalized weakness and a loss of 30 pounds in weight in the year preceding admission led him to seek medical attention. The patient's mother died at age 62 from "heart trouble." His father was alive and, though not examined, was believed to have symptoms of mild myotonia. The only sibling died in infancy.

On physical examination he was a moderately eunuchoid, thin, hatchet-faced, apathetic, apparently chronically ill male who looked young for his age. There was slight thinning of the scalp hair over the frontal region. Moderate gynecomastia and decreased axillary, pubic and facial hair were present. The penis was of adult size and the testes were atrophic, soft, and about the size of an almond. Incipient cataracts were seen bilaterally. Neurologic examination revealed weakness and atrophy of the muscles of the shoulder girdle, and generalized reduction in muscle strength, with weakness most evident in the facial and gluteal muscles and in the flexors of the foot. A pronounced myotonic reaction was elicited on mechanical stimulation of the thenar muscles and the tongue. The thyroid was not enlarged.

Pertinent laboratory studies: Urinary 17-ketosteroids were 16.1 mg. per twenty-four hours and the titer of gonadotropins less than 80 mouse units per twenty-four hours. No spermatozoa were found on examination of semen. The serum cholesterol level was 151 mg. per 100 ml. X-ray examination of the skull, an electrocardiogram and an electroencephalogram did not show any abnormalities. Electromyograms recorded from muscles of the right hand and forearm showed a myotonic pattern with evidence of atrophy. The testicular pathologic picture is presented in Table 3.

True hermaphrodism with a male chromatin pattern demonstrating an early stage of fibrosis of the seminiferous tubules in the scrotal testis and ovotestis.

D.M. (B.H. A 7889), born with multiple congenital anomalies including hermaphroditic external genitalia, Eisenmenger's syndrome with patent ductus arteriosus and a bicuspid pulmonic valve, maxillofacial dysostosis, and deformities of the ears, nose and palate, died at the age of 14 months. At autopsy he had a hypospadiac phallus bound down in chordee, behind which was a single, slit-like perineal orifice; the bifid scrotum contained two gonads each the size of an immature testis. Arising from the urogenital sinus, a blind vaginal pouch was identified which did not communicate with a rudimentery uterus. A Fallopian tube or vas deferens are not mentioned in the protocol. On microscopic examination, the gonad from one scrotal sac contained collections of seminiferous tubules in one area and primordial follicles with ova in another area. The opposite scrotal gonad was a testis containing immature seminiferous tubules. The seminiferous tubules in each gonad were lined with undifferentiated cells and spermatogonia, and many had a thickened tunica propria (Fig. 6). Cytologic sex was determined in many

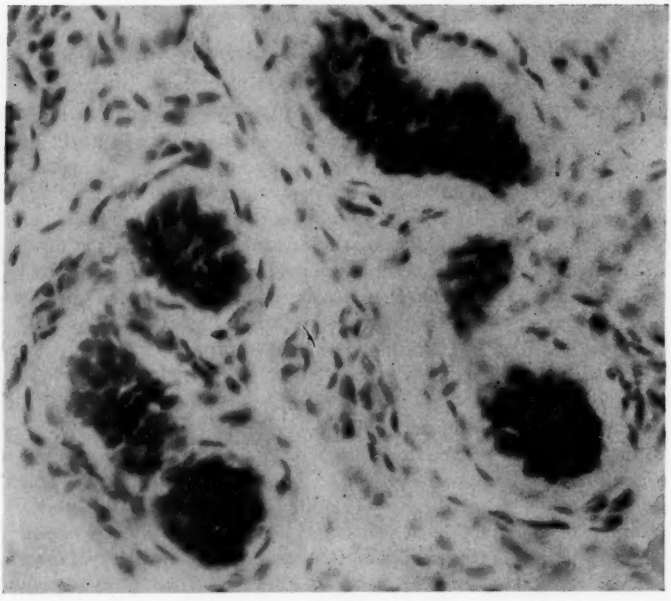


FIG. 6. Histologic section from the scrotal testis of a 14-month-old true hermaphrodite, illustrating marked thickening of the tunica propria of the small, immature tubules. Hematoxylin-eosin, 504×.

of the tissues in which nuclear detail was satisfactorily preserved. In all instances a male chromatin pattern was obtained.

Comment: This 14-month-old true hermaphrodite with scrotal ovotestis and testis, a urogenital sinus, and male-type nuclei illustrates that fibrosis of the seminiferous tubules may occur at an early age.

DISCUSSION

The detection of a female chromatin pattern in the somatic cells of some patients with seminiferous tubule dysgenesis is of practical value in the diagnosis and classification of primary testicular disease. Of particular interest is the bearing of this observation on present concepts of sex determination and gonadogenesis in the human and on the pathogenesis of certain testicular afflictions. Morever, the discordance between the sex chromatin pattern and the sex of the gonad suggests a relationship between seminiferous tubule dysgenesis in chromosomal females and the classic forms of true hermaphrodism.

Validity of the sexing technique

Pertinent to these considerations is the nature and significance of the sex chromatin mass present in intermitotic nuclei of somatic tissues in the female. Evidence that the

sex chromatin represents the fusion of the heterochromatic segments of two X-chromosomes has been comprehensively considered by Barr (19) and by Lennox (45) in a detailed, thoughtful review. This assumption is based on the morphology and staining properties of the sex chromatin body, particularly heterochromaticity (46), and its content of desoxyribose nucleic acid, the characteristic nucleic acid of the chromosomal substance (47, 48). Occasionally, the sex chromatin body appears to be double. The intranuclear mass has been identified in early human embryos (49) and cat embryos (5) prior to differentiation of the gonads, and in syncytiotrophoblastic cells of an implanting human blastocyst (49). Age, and known environmental or exogenous factors, including hormones (50), do not affect the sex chromatin. In man, the sex chromatin is found in the female, the homogametic sex (XX). This is also true for numerous species of mammals of the orders Primate, Artiodactyla, and Carnivora (4). Furthermore, in disturbances of human sex differentiation, gonadal sex, when it can be identified, corresponds to the sex chromatin pattern with two notable exceptions—true hermaphrodism and some cases of seminiferous tubule dysgenesis.

The evidence cited is of an indirect and circumstantial nature but, viewed over-all, strongly suggests that the sex chromatin mass represents an XX sex chromosome complex. It is fair also to state that no acceptable alternative hypothesis has been advanced. Nevertheless, there are obvious deficiencies in this explanation. The fact that in the male no intranuclear body can be found of a size roughly half as large as that of the female sex chromatin mass has not been satisfactorily explained, though in man the Y-chromosome is smaller than the X and not comparably heterochromatic. It is true that in cats, during chromatolysis, the sex chromatin in the neurons of females enlarges, and in the nuclei of neurons from the male a small, but distinct, chromatin mass becomes visible in about 25 per cent of the cells (51). It is also necessary to inquire why sex chromatin cannot be demonstrated in all or nearly all female cells in tissue preparations. Recent studies suggest that technical factors may be of paramount importance.[4]

Albeit in normal individuals cytologic sex and chromosomal sex are equivalent, further confirmatory studies are necessary before such an assumption can be completely accepted in applying Barr's technique to the study of disturbances of sex differentiation. Evidence in support of this assumption is already available. Hsu (54) has devised a tissue culture method which permits the study of individual chromosomes in certain somatic as well as germ cells. In dermal cells obtained from male pseudohermaphrodites, an XY sex chromosome complex was identified (55). Severinghaus (56) previously had described a male sex chromosome pattern in the primary spermatocytes of a male pseudohermaphrodite. By indirect methods, additional evidence in favor of an XY complex in patients with gonadal dysgenesis and a male chromatin pattern has been obtained recently.

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[4] Technical factors influence the results of sex chromatin counting in a variety of ways. Proper fixation and staining, type of tissue, selection of normal intermitotic nuclei, and comparison of the findings of two trained observers are all important considerations. The ideal count of 100 per cent incidence of sex chromatin in the nuclei of females is, however, not obtained in practice. This may be due to differences in the location of the sex chromatin within the nucleus (19, 45), which makes its identification more difficult in ordinary sections. The importance of high technical standards is shown by counts approaching 100 per cent in females by the use of special stains (52), or of whole mounts of tissue, such as the amniotic membrane of the cat (5) or of man (53).

female determiners on the autosomes, other interpretations of the balance theory have been proposed (62–66). Spontaneous and experimentally produced forms of ambisexual development provide evidence that this genetic balance system of male and female sex-controlling genes may be modified by various genetic, hormonal and environmental factors.

Gonadal factors

It is generally agreed that an actual imbalance in favor of either the male or female sex-controlling genes determines the sex of the gonad in placental mammals. However, Jost (68, 69) has shown that it is principally the fetal testis which controls, by its hormonal secretion, the subsequent development of the remainder of the primordial genital tract (Fig. 7). (The genes may also play a part in this process, through control of quantitative aspects of hormonal secretion and the threshold of tissue response (70).) The primary role in man of the fetal testis and of its secretion in the differentiation of the genital ducts, the urogenital sinus and the external genitalia has been discussed in a previous paper (23).

Embryogenesis. Early in its development, the embryonic gonad is composed of two unipotential, discrete, mesodermal primordia, each with a distinct physiologic, as well as morphologic, capacity: 1) *a cortical component* consisting of the germinal epithelium and 2) *a medullary component* made up of the primary sex cords and mesonephric or blastemal elements. (The origin of the constituents of the medulla is still controversial.) The sex-specificity of the medullary and cortical structures in vertebrates is a firmly-established physiologic and anatomic concept. In the human, the indifferent gonad can be identified during the fifth week of embryonic life (71–73). Its morphologic constituents are not as sharply delineated as in lower vertebrates (72). The germinal elements of the gonad are represented by the primordial germ cells, which originate in the endoderm, and in man migrate to the genital ridge at the time of early gonadal differentiation (72).

From the results of experiments in lower vertebrates (66, 74–77), particularly those of Humphrey (74), the gonocytes appear to be bipotential cells, and their ultimate development as oogonia or spermatogonia determined by cortical and medullary inductors.[6] In the absence of germ cells, sterile gonads which resemble testes are formed in both sexes. Thus, in lower vertebrates, primordial germ cells are apparently essential for normal cortical development (66, 72, 75).

Concept of dominance. The cortical and medullary tissues are heterologous structures, similar in this sense to the Müllerian and Wolffian ducts,

[6] On the other hand, Dantschakoff contends that the gonocytes are sex specific, with special affinity for cortex or medulla, and induce differential development of the bipotential gonad (78).

in that the developmental potential of each is clearly defined. The cortex can differentiate only as an ovary and the medulla only as a testis (72, 74, 75, 77–80). During differentiation, each of these elements "competes" for dominance; the dominant element conforms to the genetic sex of the zygote and the recessive element retrogresses. When the gonad begins to develop in a male direction, the cortical component involutes and only limited secondary sex-cord formation occurs (81). However, until the cortical component completely regresses, the potential for forming ovarian elements persists. The gonad destined to become an ovary develops secondary sex cords from the germinal epithelium of the cortex. In association with their proliferation, the medullary component including the primary sex cords recedes. In placental mammals the transition from a sexually indifferent gonad is relatively more rapid than in many lower species, and the recessive component is more transient and less well demarcated (72, 77, 82). Furthermore there is a disparity in the rudiments of the recessive component in the fully-developed gonad in each sex. The human ovary contains significant vestiges of the recessive medullary component, whereas none exists as such in the testis, with the exception, perhaps, of the visceral layer of tunica vaginalis.

Rate of differentiation. Evidence for an important sex difference in gonadal morphogenesis is furnished by the rate of differentiation. This applies to the appearance of visible sex differences in the gonad (72, 73), and also to the time when the pathway of future development of the indifferent gonad is finally and irrevocably fixed in each sex (77, 82–84). With the organization of primary sex cords within the medulla (in man this occurs during the seventh week), the testis presents a distinctive appearance. Specific ovarian features do not appear until later (73). The results of transplant experiments of embryonic rat gonads by Buyse (83), Moore and Price (84), Torrey (82), Holyoke (85, 86) and Macintyre (87) indicate that in genetic males future testicular differentiation is already fixed at the time the indifferent gonad can be identified. Ontogenetic fixation occurs at a much later stage in the case of the ovary (about simultaneously with the formation of secondary cortical cords), and until this occurs, transplantation may result in the development of a rudimentary testis or ovotestis, as well as an ovary.

This sex difference in the attainment of stability of gonadal organization in placental mammals, and the early predominance of the medullary component in both sexes, was interpreted by Torrey (82) as a predilection toward male differentiation of the gonad in females as well as males. In this manner, the primordial gonad begins to develop first as a testis and only secondarily undergoes modification in a female direction when secondary sex cords form and proliferate.

Extrinsic factors and inductors. Gonadogenesis may be viewed as essentially a local competitive process for dominance between the two gonadal primordia, which involves not only growth of one element but inhibition of the heterologous element (66, 72, 77, 82). The outcome is normally predetermined by the genetic sex of the zygote. Nevertheless, the balance between the medullary and cortical components can be altered contrary to the genetic constitution by many and seemingly unrelated experimental procedures, through inhibition of the dominant element. The resultant emergence of the recessive component leads to varying degrees of gonadal inversion.

Physical and other environmental factors, in addition to hormones, can induce such changes. Burns (77) has comprehensively reviewed these factors in vertebrates. In general, the higher the species the lower the incidence of spontaneous and induced reversal of gonadal development. Of particular significance are the results of embryonic gonad transplant experiments in the rat, which not only demonstrate the greater stability of the primordial testis but also suggest that these testes secrete a sex-differentiating substance capable of inhibiting ovarian development. When prospective ovaries were transplanted into castrated adult hosts of either sex adjacent to embryonic testes, "medullarization" of the ovary occurred. Various degrees of transformation were observed by Macintyre (87), including the formation of seminiferous tubule-like structures. Similar results have been reported recently by Holyoke (86) in the rabbit. These findings in placental mammals are consistent with the many previous observations in amphibia and birds[7] involving parabiosis or gonad transplantation—notably those of Burns (88), Humphrey (79), Witschi (66), Willier (75) and Wolff (89).

It is well established from the work of Gallien (90), Padoa (91), Witschi (72), Willier (75) and others that various steroid hormones can cause reversal of gonadal development in amphibia and birds. Despite similar attempts in a variety of mammals, the only convincing experimental demonstration of hormonally induced gonadal inversion has been in a marsupial, the opossum. Burns (92, 93) produced ovotestes and ovaries in male opossum fetuses by the administration of estrogen at a critical stage of gonadal morphogenesis.

Although gonadal reversal has not been achieved experimentally by use of the steroid sex hormones in placental mammals, important evidence concerning the effects of embryonic sex hormones has been obtained from the study of anomalies of sex development occurring in the freemartin.

[7] In the analysis of animal experiments it is necessary to consider species variation in the dominance of the embryonic gonad as well as other factors. For example, in birds, the female is the heterogametic sex, and the ovary is the dominant gonad.

The freemartin is a genetic female twin of a male calf (80, 94), in which the gonads and other sex structures have been modified along masculine lines.

Lillie (80) demonstrated that fusion of the fetal membranes with anastomoses between the chorionic blood vessels in the oppositely sexed twins was necessary in order for the freemartin phenomenon to occur. He further showed that such anastomoses may be present at an early embryonic stage.[8] This vascular pattern enables a hormone from the testes of the male twin to transform the gonad of the female twin into a sterile testis by suppression of the ovarian cortex (75, 80, 97). As a result of this gonadal modification the Wolffiian-duct derivatives differentiate, and in most cases the Müllerian-duct elements are completely suppressed. The external genitalia of the freemartin are usually feminine but may show some masculine development. The degree of masculinization of the genital ducts and external genitalia would appear to depend on the functional capacity of the sex-reversed gonad. It should be emphasized that, as in man, the testis of cattle develops along sex-specific lines earlier than the ovary.

Hughes (98) has described several cases of a freemartin-type twin in heterosexual pairs of pig fetuses. That this phenomenon may depend on the stage in fetal life at which the vascular anastomoses are established as well as on other factors is suggested by the failure to observe gonadal inversion when vascular connections between heterosexual twin fetuses were either observed or constructed in the cat (99) and the rabbit (68). Modification of sex development along the lines of the freemartin in heterosexual pairs of human twins has apparently not been found.[9]

The important contribution of the freemartin to the concept of gonadogenesis in placental mammals is that an ovary has been transformed into a testis, not only by an embryonic morphogenetic substance transmitted through the circulation, but in opposition to the genetic constitution of the fetus. The nature of this presumably hormonal sex-differentiating substance or inductor is unknown. Witschi (66, 72) postulated that the medulla produces a protein inductor substance (medullarin) and the cortex a heterologous inductor (corticin) which are antagonistic in their action. Burns (77), after critically evaluating the evidence, supports the notion that the gonadal inductor substances are similar although not identical to adult sex hormones.

SEMINIFEROUS TUBULE DYSGENESIS

Relationship to human gonadogenesis and sex differentiation

The present concept of gonadogenesis in placental mammals, which in principle is similar for all vertebrate species, offers an explanation for the findings in seminiferous tubule dysgenesis in *chromosomal females*. In these

[8] Additional support for the intermixing of fetal blood in dizygotic cattle twins is provided by the occurrence of blood group chimera (95) first described by Owen (96). These twins have one set of blood group antigens corresponding to their own genetic constitution and a second set identical to that of the twin.

[9] There is one documented instance of a blood group chimera in human dizygotic twins, involving an heterosexual pair (100). No abnormality of sex differentiation was observed in the female twin, nor was fertility affected.

patients, failure of the cortical component to develop and to suppress the medullary element would result in the differentiation as a testis of the primordial gonad constitutionally destined to become an ovary (Fig. 8). According to the inductor theory, this defect implies a basic disturbance in the cortical and medullary inductor mechanism whereby the medullary substance assumes a dominant role. These patients also serve to illustrate

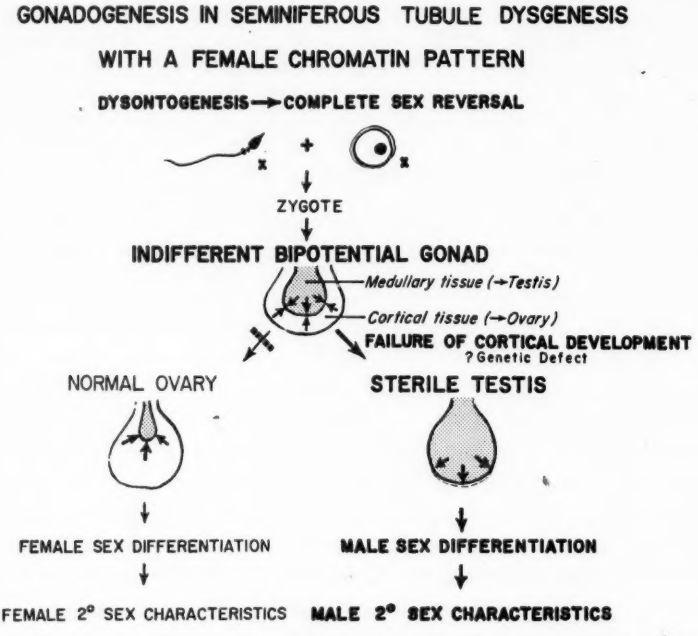

FIG. 8. Gonadal dysontogenesis and its consequences in *chromosomal females* with seminiferous tubule dysgenesis.

the latent morphologic and physiologic potential of the medullary primordia in females to develop and function as a testis.

It is not clear whether this defect in cortical differentiation involves a failure of the primordial germ cells to migrate to the genital ridge or to survive and multiply after implantation in the gonad. Primordial germ cells are not essential for gonadal differentiation in lower vertebrates, but if they are absent the gonad is sterile (72, 75, 77) and there is little or no development of the cortex. Trabucco (101) and del Castillo *et al.* (102) have postulated that the absence of germ cells in germinal aplasia of the testes may be due to failure of these cells to migrate into the primordial gonad. It is also possible in chromosomal females that the failure of cortical development is intimately related to a deficiency in primordial germ cells.

As a result of the normal hormonal function of the fetal testis in seminiferous tubule dysgenesis (Fig. 8), the genital tract differentiates as a completely masculine structure (23). The occurrence of hypospadias has been recorded but it is rare (103). Furthermore, at puberty, if the function of the Leydig cells is adequate, male secondary sex development ensues. The gynecomastia is secondary to the testicular lesion; its occurrence is not specifically related to the sex chromatin pattern.

In chromosomal males and females with seminiferous tubule dysgenesis, therefore, the defect in gonadogenesis principally involves the germinal elements. Occasional cases show some gametogenesis after careful microscopic examination of the testes. Two of our patients with female-type nuclei had spermatogonia and primary spermatocytes, in isolated seminiferous tubules. Bunge and Bradbury (104) found rare tubules with primary spermatocytes in 3 patients with Klinefelter's syndrome and a female chromatin pattern. Spermatozoa have been identified by Nelson (105) in a single tubule from 1 patient. In all patients studied, gametogenesis has been severely compromised or absent and the seminiferous tubules malformed

Thoughts on pathogenesis

Although in patients with seminiferous tubule dysgenesis and a female chromatin pattern, the sex chromatin mass may represent a more complex sex chromosome constitution (for example, XXY), it is not necessary to postulate such a chromosomal aberration. The familial occurrence of this disorder in 2 instances in our group of cases and its association with myotonia dystrophica, a heritable disease, in 1 patient, suggests that it is genetically determined. This possibility has been advanced previously by other workers (36, 41, 103). Reifenstein (103) studied a family with the Klinefelter syndrome in which 9 out of 10 male members in two generations were affected, and postulated a sex-liked recessive mode of inheritance (106).

From the available data, the exact nature of the postulated genetic defect is not clear, nor can it be ascertained that all cases are of identical etiology. The inversion of gonadal development in the female cases could be caused in some instances by a deleterious factor which results in suppression of the cortical component of the primordial gonad. Nevertheless, to affect gonadogenesis in this manner, it is apparent that the disturbance must be predetermined at the time of fertilization or take place before the sixth to eighth week of embryonic life.

It is necessary to account for two types of gonadal disturbance in these patients: 1) the reversal of gonadal development in females, and 2) the disturbance in seminiferous tubule development and gametogenesis in both sexes. There is a possibility that both lesions could be caused by a single gene defect in the sex-determining genes, arising from a mutation

or "chromosomal aberration" (63, 107, 108). It is a matter of speculation whether the fault lies in genes carried by an X-chromosome or by an autosome.[10] Indeed, the defect as well as the mode of inheritance may vary in different cases. No clear-cut sex difference in the severity of the clinical or pathologic findings is apparent in our small group of cases with tubular fibrosis. However, there is no certain method by which to distinguish chromosomal males with seminiferous tubule dysgenesis from those with similar findings in whom the etiology may be other than congenital. This deficiency limits interpretations in regard to relative sex incidence and to the variation in severity in affected males. In addition, although all patients with seminiferous tubule dysgenesis and a female chromatin pattern have been sterile, this may not prove to be invariably true. The application of Barr's technique to the study of testicular disorders will undoubtedly further clarify this problem.

Little is known of the natural history of seminiferous tubule dysgenesis. Cases of this disorder have not been diagnosed before the age of adolescence and only rarely before adulthood. As a result, the histopathology of the testes at various ages has not been described. For example, it is not known for certain if the deficiency of germ cells is congenital or acquired. This also applies to the onset of hyalinization of the seminiferous tubules. The presence of fibrosis in the scrotal testis of our 14-month-old true hermaphrodite indicates that such changes may occur at an early age. Since the degenerative changes in the seminiferous tubules are progressive and in many cases apparently delayed in onset until later life, seminiferous tubule dysgenesis has certain of the characteristics of an "abiotrophy."

Relationship of seminiferous tubule dysgenesis to true hermaphrodism

The syndrome of a masculine phenotype with normal male secondary sex characteristics or eunuchoidism, malformed seminiferous tubules, and female-type nuclei appears to be the most advanced form of a sex inversion in the human, lacking only fertility to be complete. In view of the type of defect in gonadogenesis and of the discrepancy between chromosomal sex and the sex of the gonad, these patients bear a relationship to the classic forms of true hermaphrodism.

True hermaphrodism. The classic criterion for the diagnosis of true hermaphrodism requires microscopic demonstration of both testicular and ovarian tissue or ovarian tissue and spermatozoa in the seminal fluid (60, 112). The sex chromatin pattern may be either male or female, but a preponderance of the female pattern has been observed in Barr's series (24).

[10] In Drosophila, autosomal recessive mutant genes have been reported (109–111) which transform genetic females (XX) into phenotypic males with sterile testes, and which produce sterile testes in genetic males (XY).

In these individuals with a female chromatin pattern there is apparently a corticomedullary imbalance during differentiation of either one or both ovaries which permits the latent medullary component to persist and subsequently to be transformed into testicular tissue.[11] The mildest form of imbalance in the female is manifested by cases with an ovotestis and an ovary; a more advanced type, by an ovotestis and a testis. True hermaphrodism is invariably associated with some degree of ambisexual development of the genital ducts or the urogenital sinus and the external genitalia, or both the upper and lower genital-tract structures. The extent of this dimorphic development seems to depend upon the functional capacity of the Leydig cells in the fetal testicular tissue (23, 69).

Seminiferous tubule dysgenesis. This proposed spectrum of embryonic cortico-medullary imbalance is further illustrated by seminiferous tubule dysgenesis in chromosomal females. In these individuals there is complete gonadal reversal, presumably from total suppression of cortical development. The fetal testis which differentiates is capable of completely masculinizing the genital-tract structures through the action of its morphogenetic hormone (23, 69). If in embryonic life the function of either or both testes be impaired at some period during the critical stage of development of the genital ducts and of external genitalia, ambisexual differentiation of one or both these structures would result. Although such a case has not been described, an individual of this type with bilateral testes and incomplete male embryonic sex development would have been classified previously as a male pseudohermaphrodite. The more widespread use of the nuclear sexing technique may lead to the discovery of a "*male pseudohermaphrodite*" with a female chromatin pattern.

Ovarian dysgenesis with vestigial medullary development. A third category includes an even more severe disturbance of gonadogenesis, as illustrated by the patient recently described by Greenblatt, Carmona and Higdon as "gonadal dysgenesis with androgenic manifestations in the tall eunuchoid female" (113).

This 25-year-old woman had primary amenorrhea, tall eunuchoid proportions, and clitoridal enlargement first detected at 14 years of age. The sex chromatin pattern was female. At laparotomy the appearance of the genital structures was identical to that found in gonadal dysgenesis with rudimentary ("streak") gonads, and an infantile uterus and Fallopian tubes. The histologic sections of the gonads showed gonadal stroma without ova or follicles, and with Leydig cells and a few small clusters of tubular structures at the periphery which were interpreted as "mesonephric nests." Through the kindness of Dr. Greenblatt we were able to review histologic sections of both gonads. Examination of the peripheral tubular structures revealed a morphologic appearance compatible with immature seminiferous tubules, lined by undifferentiated cells. The protocol and biopsy

[11] A similar explanation would account for the development of ovarian tissue or an ovary in true hermaphrodites with a male chromatin pattern.

specimens of a similar case with a female chromatin pattern, eunuchoidism and apparently pubertal enlargement of the clitoris, but without increased stature, were sent to us by Dr. George Clayton. In this patient Leydig cells and rete tubules were present in the gonadal stroma, but no seminiferous tubules, ova or follicles could be identified.

One can postulate that in these cases the disturbance of gonadogenesis was of such severity that in addition to complete failure of the cortical component to differentiate, development of the medullary component was defective and only rudimentary gonads (vestigial testes) were formed. Morever, as a result of deficient function of the defective medullary component, female development of the genital ducts and external genitalia ensued. The mild degree of virilization seen at puberty can be attributed to the secretion of small amounts of androgen by the Leydig cells. To distinguish this group from the syndrome of "gonadal dysgenesis with phallic enlargement and a male chromatin pattern" described previously (23), the title "*ovarian dysgenesis with vestigial medullary development*" is proposed. In the latter group, short stature and anomalies associated with gonadal dysgenesis have not been present.

Table 4 shows a postulated relationship of chromosomal females with seminiferous tubule dysgenesis to other forms of true hermaphrodism associated with a *female* chromatin pattern.

In Table 5 these various disorders are compared and contrasted.

In sum, the concept set forth of cortico-medullary imbalance of the primordial ovary and its ontogenetic and clinical consequences provides an interpretation of the anomalous developmental behavior, and serves to link the diverse clinical manifestations of these syndromes to a common basic mechanism. It delineates the stage of embryonic development during which the atypical sexual differentiation must occur, but it has no etiologic denotations.

It is apparent from this brief review that important gaps and deficiencies exist in our knowledge of many aspects of gonadogenesis, especially in placental mammals.

TABLE 4. VARIETIES OF "TRUE HERMAPHRODISM" WITH A FEMALE CHROMATIN PATTERN: A RELATIONSHIP BASED ON VARIATIONS IN CORTICO-MEDULLARY IMBALANCE DURING GONADOGENESIS

A. *Classic true hermaphrodism*
a) Ovary and Ovotestis
b) Ovary and Testis
c) Ovotestes
d) Ovotestis and Testis
B. *Seminiferous tubule dysgenesis*
C. *Ovarian dysgenesis with vestigial medullary development*

TABLE 5

	True hermaphrodism	Seminiferous tubule dysgenesis	Ovarian dysgenesis with vestigial medullary development	Syndrome of gonadal dysgenesis
Chromosomal sex (cytologic)	♀ > ♂	♀ or ♂	♀	♂ 80%
Gonads	*			
1) Medullary derivatives				
a) Seminiferous tubules	Usually defective	Defective	Absent, or rare vestige	Absent
b) Leydig cells and mesonephric elements	Variable	Present	Rudimentary with masses of Leydig cells	Rudimentary
2) Cortical derivatives; primordial follicles	Present	Absent	Absent	Absent
3) Germ cells	Present	Absent or rare	Absent	Absent
Sex differentiation				
a) Genital ducts	Variable degree of ambisexuality	Male	Female	Female
b) U-G sinus and external genitalia		Male	Female with clitoridal enlargement	Female†
Secondary sexual development	Variable	Male	None→slight virilization	None
Associated anomalies	Usually none	Usually none	None (?)	Multiple
Fertility	Fertile or sterile	Sterile	Sterile	Sterile
Familial incidence	None (?)	Sporadic	?	None (?)

* Ovarian and testicular elements present in various combinations.

† In rare cases with a male chromatin pattern and masses of Leydig cells in the genital ridge, phallic enlargement may be present.

SUMMARY

1. The sex chromatin pattern was determined in 65 phenotypic males with a variety of testicular disorders; in all but 1 patient testicular biopsy was performed.

2. Nine patients with tubular fibrosis or germinal aplasia of the testis were found to have a female chromatin pattern. In these cases the chromosomal sex and the testicular histopathologic structure indicated a gonadal disorder of a congenital nature, which has been termed *seminiferous tubule dysgenesis*. A group of 23 chromosomal males with similar pathologic and clinical findings have also been included in this category. Klinefelter's syndrome was a manifestation of this condition in some cases; however, not all patients with Klinefelter's syndrome had seminiferous tubule dysgenesis. The disorder was familial in 2 instances. One patient had myotonia dystrophica. Criteria for the diagnosis of seminiferous tubule dysgenesis are discussed.

3. Present knowledge of gonadal ontogenesis in vertebrates was reviewed, particularly in placental mammals. In the light of animal experiments, the failure of the embryonic gonad to differentiate as an ovary in chromosomal females with seminiferous tubule dysgenesis has been attrib-

uted to a severe cortico-medullary imbalance with complete suppression of the cortical component (ovarian primordium). As a result, the embryonic gonad is transformed into a testis capable of bringing about male sex differentiation. The evidence, though inconclusive, suggests that seminiferous tubule dysgenesis is a genetically determined disorder.

4. Seminiferous tubule dysgenesis in chromosomal females appears to be the first example of complete sex reversal in the human. According to the theory presented, these individuals may be regarded as a variant of classic true hermaphrodism because of the discrepancy between chromosomal and gonadal sex and in view of the mechanism of gonadal inversion. A concept of true hermaphrodism is proposed based on varying degrees of cortico-medullary imbalance of the embryonic gonad.

5. The role of cytologic diagnosis of sex in the investigation of primary testicular disorders is discussed. This procedure should become a routine part of the study of testicular histopathology. Detection of a female chromatin pattern in phenotypic males is presumptive evidence of seminiferous tubule dysgenesis, provided true hermaphodism and rare forms of female pseudohermaphrodism can be excluded. A male chromatin pattern in patients with primary testicular disorders does not rule out the diagnosis; in these individuals it must be based on the testicular pathologic picture. Determination of the cytologic sex of boys with unusually small or firm testes, with cryptorchidism (unilateral or bilateral), or with adolescent gynecomastia should lead to the discovery of prepuberal cases and provide the opportunity to study the testicular lesion in this disorder at an early age.

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Addendum

Additional reports of observations in individuals with seminiferous tubule dysgenesis have appeared since this paper was written (114–116).

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GENETIC, DEVELOPMENTAL AND HORMONAL ASPECTS OF GONADAL DYSGENESIS AND SEX INVERSION IN MAN*†

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ABNORMALITIES of sex in man, as seen in clinics and extensively described in the medical literature, show a great variety of manifestations which have long defied attempts at rational classification. However, the discovery by Barr (1) of a sexual dimorphism in the chromatin of resting cell nuclei now permits a clear separation of the cases involving sex inversion from those that merely present forms of under- or over-development of sex organs (2). In the present discussion we shall speak of *sex inversion* and of *contrary sex development* whenever the appearance of gonads or somatic organs obviously conflicts with the chromosomal or constitutional sex. Complete sex reversal including the capacity for reproduction may occur spontaneously and can be produced experimentally in fishes and amphibians (3, 4) but, so far, is not known in any of the higher vertebrates. Yet, the observation of full spermatogenesis in an individual with female sex chromatin (Fig. 5) indicates that, even in man, functional sex reversal lies within the range of possibility.

Studies in the etiology of sex inversion in man lead us to recognize three definite classes in regard to causation and developmental type. Each has its specific teratogenic set-up. Evidently the outcome of abnormal development must depend not only on the nature of the teratogenic agent but also on the time at which it begins to interfere with normal progress. The development of the sex organs passes through four major periods (Fig. 1): *the undifferentiated stages* (St. 1–30), *the period of gonadal differentiation* (St. 31–34), *the period of prenatal differentiation of secondary sex characters* (St. 34–36 to birth), and *the puberal period.* Every one of these divisions includes the starting points of a characteristic class of sex inversions.

Since the possibilities of experimental analysis of sex determination in

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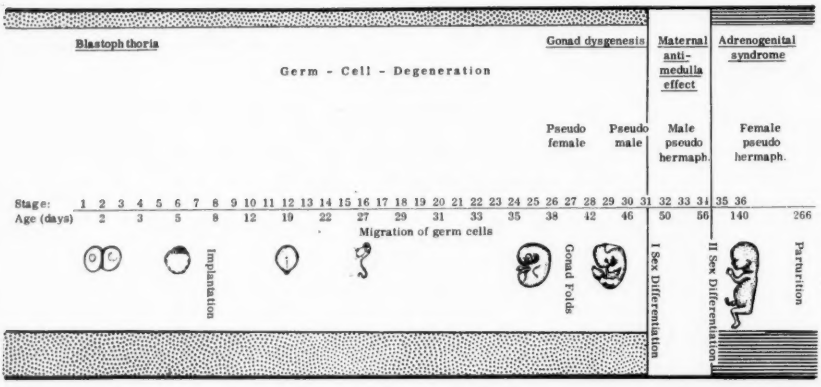
Blastophthoria
Germ - Cell - Degeneration
Gonad dysgenesis
Maternal anti-medulla effect
Adrenogenital syndrome
Pseudo female
Pseudo male
Male pseudo hermaph.
Female pseudo hermaph.
Stage: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36
Age (days) 2 3 5 8 12 19 22 27 29 31 33 35 38 42 46 50 56 140 266
Migration of germ cells
Implantation
Gonad Folds
I Sex Differentiation
II Sex Differentiation
Parturition

FIG. 1. Location of the teratogenic stages of three classes of sex inversions in human development.

Stippled: Stages 1–31: indifferent period.
White: Stages 31–34: period of primary sex differentiation.
Striped: Stages 35, 36, to birth: period of secondary sex differentiation (accessory sex organs).
Standard stages after Witschi 1956 (34).

man are very limited, reference will be made to animal experiments which may contribute toward an understanding of the causality of sex inversion in each of the following three classes.

I. THE ADRENOGENITAL SYNDROME

It is well known that the *sequence of aberrant developments* represented under this name starts with a general or tumorous hyperplasia of the adrenal cortex. This primary abnormality, by production and release of androgenic steroids, induces the characteristic depression of gonadal function and concurrent development of male secondary sex characters. In male individuals the result is merely a sort of somatic sexual precocity. However, in females the subsequent course of sexual differentiation veers from the feminine toward the masculine type. It is a significant fact that the manifestation of this syndrome may begin at various age levels, but never before the fifth month of gestation.

This explains the peculiar variety in morphologic appearance of the resulting *female pseudohermaphrodites.* In fetuses of 120 days, the gonaducts already have assumed a female type, though the mesonephric ducts may not have disappeared completely, and the uterovaginal rudiment is still attached to the upper dorsal wall of the urogenital sinus. Hence, the earliest and most extensive masculinizations lead to a type in which there are normal tubes and uteri, but in which the vagina opens into the urethra. Along-

side the uterus persist minor remnants of mesonephric ducts, and parts of the urethra are surrounded by prostatic tubules. The external genitals manifest considerable development; particularly, the phallus may grow to such a size that at birth the child often is mistakenly pronounced a boy. However the organ is ventrally open, and the urogenital orifice lies at its base (Wilkins (5) mentions one "rare exception"). This characteristic hypospadias is a direct consequence of the late (fifth month) onset of teratogenesis. In normal male development, closure with raphe formation starts early (two and a half months) and is completed well before the end of the fourth month.

Dispelling all possible doubt concerning the genetic sex of congenital female hermaphrodites, the study of the cell nuclei in 56 cases led to the recognition of the female chromatin pattern in every instance.

In the cases of very late onset, at puberty or later, manifestations are restricted to hypertrophy of the clitoris, hypertrichosis and change ofvoice —often collectively designated as *virilism in the female.*

The highly *familial distribution* of masculinizing adrenal hyperplasia points toward some form of heredity. Typically, in large families, some of the siblings are normal, others affected. Wilkins (5) reported a family with 4 children—2 precocious boys and 2 daughters, of which one was normal and one a pseudohermaphrodite. In a recent case observed at the Iowa University Hospitals, the male member of a pair of fraternal twins was a normal boy and the female was a pseudohermaphrodite (patient of Dr. W. C. Keettel: K. H. #55–9538; 4 years). As far as known, the parents of such families were normal in every instance. This would almost be imperative, since affected individuals are unable to reproduce. Obviously the hereditary defect is of the nature of a recessive gene, possibly with a series of quantitative alleles. Polygenic inheritance should also be considered. Since the milder forms of the syndrome (virilism) probably have a similar genic background, it may be expected that the modern methods of restoring fertility by hormone administration (6, 7) will result in an increase of the incidence of this sex deviation.

In the fetus, the onset of abnormal adrenal development coincides with, or follows, the rapid rise of estrogens to the high levels that prevail during the second half of pregnancy. Virilism likewise is reported to manifest itself often under, or following, conditions of high estrogenization, such as puberty or gravidity. Whether or not a direct cause-and-effect relationship between estrogen levels and pathologic development of the adrenal actually exists deserves further critical examination. It is noteworthy that in experiments with amphibian larvae the administration of large dosages of estrogens produces an excessive tumorous growth of the adrenal cortex (8, 9). Chromatographic analysis shows that animals with such tumors release

quantities of one or two 17-ketosteroids which do not occur normally (Chang, Chieffi, Witschi, unpublished).

Recent studies lead one to assume that the gene mutation underlying the development of congenital adrenal hyperplasia is related to an adrenal factor, possibly affecting one of its enzyme systems. As a consequence of this metabolic defect, androgenic steroid production is enhanced while corticoid synthesis is impaired, so that hypophyseal release of adrenocorticotropin continues unchecked. The observation of high production of adrenocorticotropin in this syndrome (10) suggests also an alternative interpretation, based on genetic modification of hypophyseal response to blood estrogen levels.

II. HEREDITARY MALE PSEUDOHERMAPHRODISM

The male pseudohermaphrodite is a genetic male with testicular sex glands and the male sex chromatin pattern, but with female or mixed male and female secondary sex characters. The teratogenic agent becomes effective immediately following primary sex differentiation, *i.e.*, early in the third month. In the most extreme cases the testes remain abdominal and lose control over the differentiation of the accessory sex organs before the end of the tenth week. Consequently the gonaducts and external genitals assume the female pattern (5). Not even an epididymis or a rudiment of a deferent duct is formed. The question of possible estrogen production during the fetal period remains undecided, since it is now known that the mammalian genital tract assumes the female structure by self-differentiation, even in the absence of gonads (11, 12). However, at four months (fetus of 10 cm.) some indications of androgenic activity appear. The vagina retains its attachment on the urogenital sinus, and the clitoral enlargement which is observed at birth also dates back to this period. The masculinizing influence obviously is weak and probably transitory, for no noticeable prostatic growth has been reported (in this respect the present cases differ from the otherwise similar early adrenogenital syndrome).

The described extreme type is relatively rare. More often the inductive deficiency of the testis is less pronounced and manifests itself at later stages. Consequently one observes a full series of compromises between male and female differentiation. Beginning at the epididymal end, the male gonaducts become gradually better developed while, of the female parts, the vagina is often the only one maintained. There are various degrees of descent of the testes. The phallus is always of clitoridal type. The development of the various sex accessory structures obviously is an accumulative expression of masculinizing influences at all periods. There is no suggestion of an alternation of masculinizing and feminizing periods, though in the course of time the balance of the competing influences may be subject to some fluctuation. Starting from the "female with testes," one might

describe any number of types with stepwise increasing prevalence of male characteristics. The last one short of the anatomically normal male is a hypospadiac with fully descended scrotal testes. It is now clear that the 2 "girls" described in 1942 by Witschi and Mengert (13) are of this type.

After puberty, there is evidence in all cases of normal or slightly increased gonadotropin levels and of estrogen production, although the hormonal picture is notably variable (14). Hormonal assays in a limited number of cases and the histologic condition of the epididymis, when present, indicate that these testes secrete androgen also. Since castration is immediately followed by disappearance of the estrogens and an increase of gonadotropin (FSH) secretion (13, 15), it is reasonable to assume that the estrogenic hormone which accounts for puberal development is produced by the testes.

Male hermaphrodites often get married to male partners (13, 14). Their insistence on maintaining the legal status of women probably is prevailingly determined by their upbringing as girls. However a systematic study of the influence of particular hormonal conditions on the development of psychic and behavioristic patterns in this group should prove of great interest.

The *mode of inheritance* of male hermaphrodism is better known than that of any other sex deviation. Unfortunately, in recent years complete family histories covering more than one generation have not been published. Adding the families reported by Mishell (15) and Stern (16) to the older cases summarized by Witschi (3), the sex distribution is *69 females, 64 hermaphrodites and 16 males.* Only 1 of the "males" is said to have had offspring; probably none was carefully examined. It seems therefore permissible to say that typically the families consist of equal numbers of women and hermaphrodites. The apparent numerical prevalence of the *hermaphrodite+male* class is the expected result of selection in this sort of compilation of cases. That the hermaphrodites took the place of men is confirmed by sex-chromatin tests (19 cases) which, so far without exception, revealed the male pattern. The study of the family trees (17, 18) further showed that half of the married daughters had normal children, whereas the other half were conductors of the deficiency, *i.e.*, they again gave birth to male hermaphrodites (3). These facts indicate that the syndrome is transmitted by a dominant gene. Mothers heterozygous for this factor deliver hermaphrodites instead of boys. Since only half of the genetically male children may actually carry the gene themselves, it is evident that manifestation depends on the constitution of the mother. In other words, some agent that interferes with the normal function of testes must pass from the mother into the fetus (Fig. 2). Speculating about the possible nature of the inhibiting agent, one is led to compare the relationship between

mother and male fetus with that of a large female salamander in parabiosis with a small male. In this combination the large female gains partial control over the sexual differentiation of the male, transforming it into a hermaphrodite (19). Extending this analogy, it is tentatively assumed that the maternal organism responds to the differentiation of fetal testes by the production of an *antagonist* which suppresses the male inductive and endocrine capacities of the testes. The acting principles of this system must be able to pass the placental barrier like some antibodies, for instance those of Rh and Hr antisera. It is not likely that estrogenic hormones are the cause of the testicular deficiency. It dates from the endocrinologically dull period, when the chorionic gonadotropins fall to a low level and the estrogens have not yet increased. Postulation of a precocious rise of estro-

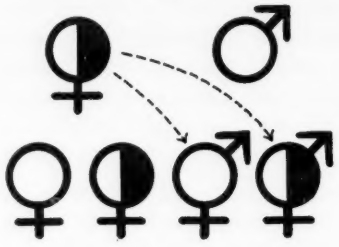

FIG. 2. Relationship of inheritance and maternal influence in the determination of male pseudohermaphrodism. *Black half discs* represent heterozygous carriers of the mutant gene. *Broken arrows* indicate transplacental transmission of an antimedulla factor from mother to sons.

gen production would present many new difficulties. If on the other hand the syndrome depended on hereditary constitution of the children, only half of the boys should be abnormal. The circumstances of the case, particularly the fact that the disturbance shortly follows testicular differentiation and affects specifically the male-inductor component, are more in favor of the proposed immunologic interpretation.

The antagonistic principle does not impair the germ cell component of the testes, which up to the age of 11 years retain a healthy appearance histologically (Fig. 3). After puberty, the seminal tubules usually contain only reduced numbers of spermatogonia and few spermatocytes, if the testes are retained in the inguinal region (Fig. 4). The later stages of spermatogenesis are suppressed, as in all cryptorchid gonads; but it is more remarkable that also in scrotal testes spermatogenesis is incomplete (13).

Male pseudohermaphrodism differs from its female counterpart by the nearly constant time of onset of the aberrant course of development. The variability of the morphologic picture is mostly an expression of the quantitative severity of the upset and not of the time of its initiation.

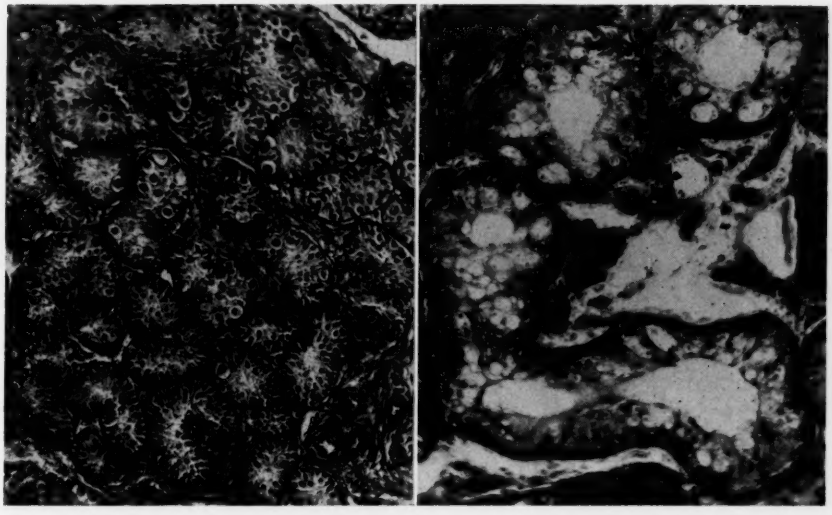

FIG. 3 FIG. 4

FIG. 3. Case K. S., No. 55-10183 (Dr. W. C. Keetel); male pseudohermaphrodite, 11 years old. Section of testis ×120.

FIG. 4. Case B. D., No. 43-1239 (Dr. W. Brown); male pseudohermaphrodite, 16 years old. Section of testis ×120.

III. PRIMARY DYSGENESIS OF THE GERM PLASM (PSEUDOFEMALES AND PSEUDOMALES)

The third class is characterized by complete or partial sterility, which is acquired before the stages of primordial gonad formation (Fig. 1). Therefore it does not include the sterility cases brought about by secondary germ cell destruction through infectious disease, radiation or other damaging conditions, during fetal or postnatal life. The individuals concerned may have the general appearance either of females or of males. However, if investigated by the sex-chromatin method, a majority of the first type, exhibiting the so-called Turner syndrome, are revealed as of male genetic sex (20, 21, 22) and therefore are *pseudofemales*. On the contrary, individuals of the second type, clinically recognized as cases of the Klinefelter syndrome, most often have the female sex chromatin pattern and then must be considered as *pseudomales* (22, 23, 24).

Of the 98 pseudofemales and 54 pseudomales whose records were available to us (2), many are married in conformity with their somatic sex, but wrongly according to genetic constitution.

The *endocrine picture* differs strikingly from that of normal men and women. As a rule both types have high postpuberal levels of gonadotropin

(20). Pseudofemales, like true agonadal women and castrates, show little evidence of steroid sex hormone production. On the contrary, pseudomales maintain more or less effective, though low, and fluctuating levels of estrogens and androgens. Although these levels account for the varying degrees of stimulation of male and female secondary sex characters, in the aggregate they are not sufficient to reduce gonadotropic output to normal proportions. The urinary output of 17-ketosteroids usually is close to the average for normal men (24, 25).

At birth and during childhood *pseudofemales* are not distinguishable from normal girls. They appear in the clinics, because at puberal age the menses fail to become established or their short stature becomes a matter of concern. Their pubic hair is sparse or absent, the breasts undeveloped, the pelvis narrow and the voice unbroken. Laparotomies reveal the absence of ovaries; at their normal site only a scar-like ridge is found.

Pseudomales throughout childhood appear in every respect like normal boys. At the age of puberty their breasts often enlarge to the extent that medical advice is sought. Other cases are discovered in sterility clinics. Beard and pubic hair are frequently scanty, and the latter may be of female distribution. The voice sometimes is rather high, though not feminine. The testes consistently are small. Usually prostates, epididymides, and deferent ducts are palpable. Semen samples have never been reported to contain sperms.

Biopsies of the testes as a rule show dense nodules of interstitial cells and small seminal tubules. In the latter, spermatogonia and primary spermatocytes may occur; at least in 1 case, all stages of complete spermatogenesis were observable in several tubules (Fig. 5). However, more commonly the tubules are sclerosed or contain only sustentacular cells (of Sertoli) either differentiated or undifferentiated (Fig. 6).

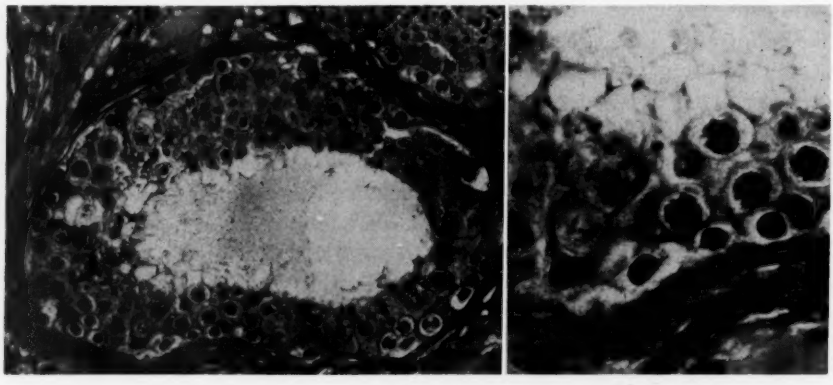

FIG. 5

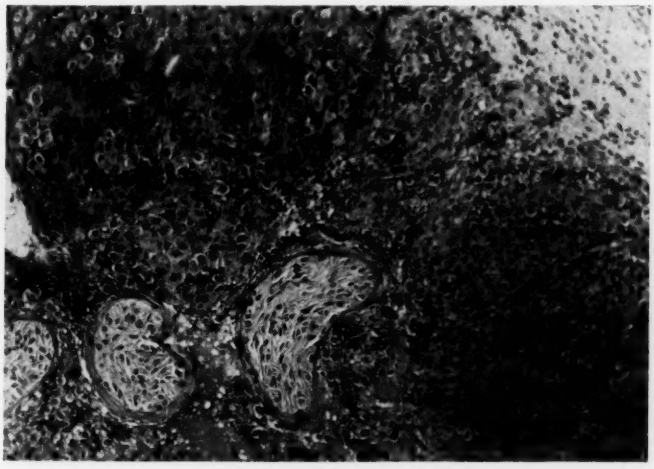

FIG. 6

FIG. 5. Case R. W., No. KM 563 (Dr. R. Leach, Detroit); pseudomale, 18 years old. Biopsy section; left ×240, right ×640.

FIG. 6. Case J. M., No. 53-6812 (Dr. R. G. Bunge); pseudomale, 26 years old. Biopsy section ×100.

An *interpretation of the etiology of pseudofemales and pseudomales can be offered on the basis of experiments with amphibian eggs.* Delay of fertilization beyond the normal term results in degradation of the ovulated egg (26). At 18° to 20° C. mature ovulated eggs develop normally if fertilized within three days. If stored longer they gradually become overripe and die after about the sixth day. Fertilized overripe eggs develop abnormally. Even if only mildly affected, the germ plasm proves highly susceptible to the damage. The primordial germ cells exhibit degenerative features, multiply slowly, and enter the gonadal folds only in small numbers or not at all. In the latter case the animal remains permanently sterile. If, however, just a few gonia persist and reach the gonad primordium, the animals become males irrespective of their genetic constitution. The decisive factor may be the correspondingly low number of follicle cells; for they constitute the active element of the cortex, *i.e.*, the ovarial inductor system. As under many other experimental conditions, the weakening of the cortex is followed by a medullary compensatory development that leads to testicular differentiation. In cases of minor damage the competition between the two systems may lead to bisexual conditions. Ovotestes sometimes persist even to the reproductive age. In one instance, a partially sex-reversed, genetically female toad was successfully bred with a normal female. Although actually a hermaphrodite with ovarial and testicular gonadal lobes, the

animal functioned as a male (27). Often the masculinizing effects of overripeness are localized—either restricted to one side, or to one segment of one or both gonads (28).

The close similarity between gonadal development resulting from experimental overripeness in amphibians, and human types of partial or complete germinal dysgenesis is evident. It is known that a majority of ovulated human eggs are of so low quality that they are incapable of normal development (Lit. 26). Various conditions attending ovulation, fertilization, and preimplantation development may cause delay and create an environment that is unfavorable for unattached eggs and blastocysts. Experiments with amphibian eggs have shown that carbon dioxide poisoning is an essential factor in bringing about the degradation due to overripeness (26). Moreover, no indications have been found that gonadal dysgenesis in man might be a hereditary type of abnormality. Therefore it seems justified to assume, at least tentatively, that germinal degradation (blastophthoria) is the primary cause of the deficient number of primordial germ cells.

Gonadal insufficiency may be but one of a series of congenital abnormalities occurring in the same individual. In Turner's syndrome such defects as coarctation of the aorta, webbing of the neck, cubitus valgus, visual disturbances, and short stature are frequently observed. Since there is no indication of hereditary transmission of these components they serve to support the idea that acquired blastemic degradation is their common cause.

If absence or a low number of primordial germ cells is the intermediary cause of abnormal primary sex development, it remains to be investigated to what extent such deficiency can explain the wide range of observed facts. The question of *sex ratios* deserves first consideration. Obviously there is no reason to expect that male and female primordial germ cells should be damaged at different rates. Hence, it seems surprising that all investigators report a large preponderance of genetic males among the "agonadal women" and of genetic females among the "Klinefelters." According to Nelson and Boccabella (2) the respective counts are 87:16 and 49:13. However, in both instances the smaller groups are of the chromosomal sex that agrees with the general external appearance of the individual. Therefore, they are not clearly or indeed not at all limited toward the large classes of individuals of the same chromosomal type with low fertility.

This point is well illustrated by a group of amenorrheal women, reported by Carpentier (21). Among the total of 35 patients, 10 were proved to be pseudofemales on the basis of a chromatin study of the vaginal smears. The remaining 25 were chromosomal females. Of these, only 2 were typically agonadal women (proved at laparotomy), so that the male to female ratio was 5:1. However, the remainder of this group consisted of 2 with

rudimentary ovaries, 6 with normal ovaries but rudimentary vaginae, and 15 with no particular symptoms besides various grades of amenorrhea. The cases necessary to bring the sex ratio to unity are probably to be found in this graded series with defective ovarian function. The remainder of the 25 genetic females would represent amenorrheal conditions developing from adrenal or hypophyseal dysfunction.

This analysis leads to the recognition that in some instances rudimentary gonads of genetic females were still able to develop into ovaries. However, this recovery was obviously incomplete. A limited resumption of ovogonial multiplication may have occurred during the fetal stages (third to tenth months). So far no case of secondary development of gonadal rudiments into ovaries has been observed in pseudofemales (chromosomal males). It appears that recovery depends on a female genic constitution.

An analogous situation pertains to the proportion of chromosomally female and male "Klinefelters." The latter are simply males of impaired fertility; on clinical grounds the diagnosis is Klinefelter's syndrome, but there is a male chromatin pattern. Actually, these are cases in which tubular fibrosis has destroyed the seminiferous tubules. In some instances this is the result of a recognized or unrecognized orchitis, but it is not unlikely that others are cases in which primordial degeneration of the germ cell may have been a factor in the later impairment of testicular structure and function.

Our discussion shows that in this class only the pseudofemales and pseudomales are distinct types. Females in whom there is no sex reversal and males with reduced numbers of germ cells are not clearly separable from cases of secondarily reduced fertility.

The *relationship between the number of primordial germ cells* and *sex differentiation* has already been mentioned. In some way the establishment of cortical and medullary organizers depends on the number of primordial gonia in the indifferent sex glands. Their frequency determines quantitatively the development of follicle cells; it also affects growth and inductive activity of the medulla. Although it would seem conceivable that in case of complete absence of gonia the medulla might enlarge and differentiate into a sterile testis, this obviously does not occur. In cases of clitoral enlargement, such as reported by Wallart and Roessle (29) and by Grumbach *et al.* (20), the rudimentary gonadal ridges contain some distinct, though not extensive mesonephric (epididymal) and medullary elements. On comparative grounds it seems probable that at embryonic stages some germ cells had been present. The latter authors (20) also describe an 8-year-old child with distinctive lateral dimorphism, that seems particularly revealing. The considerable size of the phallus had caused doubts as to the true sex. The investigation exposed a completely sterile gonadal rudiment and an oviducal tube on the left side. On the right a slightly smaller rudiment con-

tained "a few scattered clusters of immature seminiferous tubules in the medullary region." The photographs seem to leave no doubt about the presence of some spermatogonia. No tube, but a deferent duct was present on this side. Nuclear tests showed the male chromatin pattern in tissue samples from selected places on both side of the body. The possibility of a chromatin mosaic thus is excluded. On the other hand, the correlation between presence of germ cells and male differentiation seems significant, since other circumstances are as nearly identical on both sides as might be ever possible. This case can be classified as a pseudofemale with partial manifestation of the genetic, *i.e.*, the male sex.

In pseudomales with descended testes the study of a single biopsy sample usually shows only sterile tubules (Fig. 6). However, in a few instances a more systematic search has been made, with the finding of occasional tubules with spermatogonia, spermatocytes, spermatids (30) and even spermatozoa (Fig. 5). Since no complete studies by serial sections have yet been made, the possibility remains that all testes contain some germ cells. So far only clinical cases past the tenth year of age have been under observation, earlier stages being difficult to find. But even the presently available data lead to the conclusion that the testes of pseudomales probably arise from gonadal primordia which contained a considerable, though subnormal number of gonia.

If the number of primordial gonia approaches the threshold for normal development, an uneven distribution of the germ cells must be expected to cause differentiation of *ovotestes* and *lateral hermaphrodism* in individuals of female genetic sex. Whereas relatively well supplied regions of gonadal folds pass normally through ovarial differentiation, the most depleted parts can be expected to follow the trend seen in the pseudomales, *i.e.*, testicular development. It is evident that in embryos of male genic constitution similar gonial irregularities would be compatible with entirely testicular differentiation, although fertility might be reduced.

The medical literature contains reports on about 60 cases of "*true human* hermaphrodites," most of which probably belong in this particular group. The lateral hermaphrodite of Greene *et al.* (31) has been studied not only morphologically but also in regard to sex chromatin patterns. According to the expert opinion of Professor M. L. Barr, sections of breast epidermis, ovary, and testis (interstitium) all showed the female nucleolar arrangement (32). This case is of particular interest because the individual produced mature sperms as well as eggs.

Whereas in Greene's case testicular and ovarial parts were almost equally well developed, all degrees of mixture can be found between gonads of typical pseudomales and ovaries of normal women. A woman with a single testicular nodule in one ovary was described by Brewer *et al.* (33), and in

TABLE 1. RELATIONSHIP BETWEEN NUMBER OF SURVIVING PRIMORDIAL GONIA AND TYPE OF SEXUAL DEVELOPMENT. PERCENTAGES ESTIMATED ON THE BASIS OF OBSERVATIONS IN AMPHIBIAN EMBRYOS

Surviving gonia, %	Ontogenetic differentiation of	
	XX genotypes	XY genotypes
0– 5	Agonadal women, permanent amenorrhea	Pseudofemales
5–15	Pseudomales and true hermaphrodites	Very-low-fertility men
15–40	Amenorrhea and low fertility	Low-fertility men
40–100	Normal women	Normal men

one of our pseudomales the ovarial cortex was represented by a very small fragment and some peculiar intersexual cords, caught within the albuginea (25).

The indifferent gonadal primordia of a human embryo of five weeks contain normally about 2,000 gonia. The number is variable, but there is a steady increase. In Table 1 and in Figure 7 the relationships between germ cell numbers and types of sex differentiation are summarized on the basis of the present analysis of human cases and the aforementioned amphibian studies. The percentages of surviving germ cells (Table 1) are only rough estimates. Actual counts of gonia would be difficult to collect, even in amphibian embryos. But it is obvious that their numbers total between 0 and 100 per cent of the normal average of a given species, and reflect the degree of damage suffered by the germ plasm.

Besides the mere *number* of gonia, certainly also their *vitality* may exert an influence on the course of gonadal differentiation. The studies on over

FIG. 7. Diagrammatic cross sections through primordial gonad folds. *Left:* normal case with well balanced cortical and medullary rudiments. *Center:* reduced number of germ cells affecting mainly the cortical component. *Right:* extremely reduced number of germ cells, affecting also the medullary component. The consequent prospects of differentiation, according to either XX or XY constitution are listed under each diagram.

ripeness show various degrees of impairment among germ cells that became included in gonadal folds. Moreover, in the development of ovotestes the *damage suffered by somatic components* of the cortical and medullary rudiments visibly influences the distribution pattern of ovarian and testicular parts (28). Distinctly the germ cells are not inductors of sex differentiation; but they have some influence on the development of the follicular and interstitial-cell elements and their inductive functions. In amphibians it may be seen that in the absence of all germ cells, gonadal folds do not differentiate into sterile ovaries or testes, but retain permanently a rudimentary state. The topography of the gonadal remnant in "agonadal women" supports the contention that the same principles apply to the development of sex glands in man. In all probability completely sterile testes or ovaries in children and adult individuals contained some germ cells at the stage of embryonic sex differentiation, and lost them only secondarily through degeneration.

DISCUSSION

After the discovery of the chromosomal mechanism of genic sex determination at the beginning of the present century, possibly the most surprising outcome of research in this field is the realization that, given the male or the female chromosomal constitution, any individual still may develop into either a male or a female. In experiments with Xenopus it was found that the genic determination of male differentiation may be upset and reversed during a sharply limited short stage of embryonic development. The pseudofemale, without changing chromosomes or genes, subsequently ripens eggs, reproduces, and behaves like a normal female for the entire life duration (4). Although only one or two years ago most experts were still doubting that experiments with lower animals might bear on the interpretation of unusual sex conditions in man, we are now confronted with startling evidence of actual *sex reversal in man.* It is derived from the analysis of nature's experiments which fall into three easily recognizable classes (Table 2). The operative agents of the first two, becoming active after completion of gonadal sex differentiation, effect morphologic reversal of secondary sex characters only. The third class alone presents evidence of very early interference with the normal course of development and leads to true gonadal sex reversal. The masculinization of both gonads in our Subject R. W. (Fig. 5) and of the right one in Greene's hermaphrodite was morphologically complete. The condition may even more adequately be designated as *XX-male* than pseudomale. Like breeding-frog hermaphrodites (34, p. 160), human pseudomales are homogametic; hence, their semen, if able to fertilize, should produce only female offspring.

In all sufficiently investigated human cases, gonadal sex reversal was

found to have taken the female-to-male direction. In animal experimentation sometimes inversions can be induced in both directions, but seldom with equally good success. The opposite results are obtained by the application of basically different experimental methods.

The occurrence of spontaneous true sex reversal in man is assurance that the experimental production of XX males in mammals must be possible in principle. However the relative inaccessibility of embryonic stages preceding sex differentiation offers serious technical obstacles to work on this subject.

For the analysis of the human cases of abnormal sexuality the identifica-

TABLE 2. ONTOGENESIS IN THREE PREVAILING CLASSES OF HUMAN SEX ABNORMALITIES

Developmental cause	Resulting sex type in	
	XX individuals	XY individuals
Adrenal hyperplasia	Female pseudohermaphrodites	Precocious puberty in boys
Maternal antimedulla reaction	Normal women	Male pseudohermaphrodites
Dysgenesis of germ plasm		
a severe	Agonadal women	Pseudofemales
b moderate	Pseudomales	Partially sterile men
c light	True hermaphrodites	Low-fertility men

tion of a *sex chromatin dimorphism* by Barr and his collaborators has become of greatest importance. Not only does it aid in the diagnosis of the genetic sex but it also renews the cytologist's interest in mammalian chromosomes. One may expect that further work will lead to the discovery of chromosomal mosaics, polyploidy, and aberrant chromosome combinations. At the present time there is no good reason to assume that unusual chromsome combinations play an important role in the causation of the more common human sex aberrations; for it has been shown that animal experiments provide satisfactory analogues and explanations for the various types of abnormalities and furnish clear evidence that sex inversions do not depend on chromosomal changes. As in Drosophila and some plants, the study of chromosomal aberrations in mammals might contribute to the understanding of the location and of the physiologic qualities of the sex-determining genes.

SUMMARY

Analysis of sex abnormalities involving sex inversions in man leads to distinction of three classes. They differ in causation, time of onset of man-

ifestation in ontogenesis, and characteristic type of adult expression. Class I abnormalities originate from dysgenesis of the primordial germ cells—itself a consequence of damage to egg or free blastocyst (blastophthoria). In the case of complete disappearance of primordial gonia preceding the period of gonadal sex differentiation, genetic males become *pseudofemales*. If the number of gonia is only substantially reduced, genetic females develop into either *pseudomales* or *true hermaphrodites*. Class II abnormalities rest on a hereditary peculiarity of mothers which makes them counteract the normal medullary functions of fetal testes immediately following their embryonic differentiation. Hence, genetically male fetuses develop into *male pseudohermaphrodites*. Class III abnormalities develop as a sequel to adrenal hyperplasia, with production of androgenic hormones. Manifestation begins only in the fifth month or later. Genetically female individuals develop into *female pseudohermaphrodites*.

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EFFECT OF LARGE DOSES OF INTRAVENOUSLY ADMINISTERED PROGESTERONE ON GONADOTROPIN EXCRETION IN THE HUMAN FEMALE*

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INTRODUCTION

ALTHOUGH a large and respectable body of literature has arisen on the relationship between the activities of progesterone and pituitary gonadotropin[1] in experimental animals, the available information on this relationship in human subjects is comparatively scanty. Funke (1) (cited by Smith and Albert(2)) and Büttner and Trappmann (3) found no significant effect of progesterone on the levels of excreted gonadotropin. The doses used by the latter authors were 10–20 mg. per day, with a total dose of 60 mg. in one case, and 120 mg. in another. Laroche, Simonnet and Bompard (4), using doses ranging from 6 to 65 mg. per day injected over periods ranging from five to twenty-three days, claimed to show a slight reduction of gonadotropins in the urine of castrated or menopausal women. Recently Smith and Albert (2) reported that progesterone in a dose of 50 mg. per day orally for eight days in one case, and Pranone® in a dose of up to 100 mg. per day for one week in another case failed to suppress the excretion of gonadotropins. Both their patients were castrated women.

In contrast, animal work has clearly shown that administration of progesterone to cycling females can lead to delay or advancement of ovulation time (5–9), to suppression of the cycle (10–16), and to a decrease in the high level of gonadotropin activity that is usually associated with castration (17, 18).

When the opportunity presented itself to give large doses of progesterone by the intravenous route to a group of patients with carcinoma of the

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[1] *Tropin* is the form used by the Journal. The author favors *trophin*.

cervix (19), we decided to follow gonadotropin excretion in these patients, in order to see whether any further light could be shed on this problem. However, since this was not the primary objective of the study, urinary gonadotropin assays could not be carried out daily. Instead, spot checks of the levels were made at arbitrary times before, during and after treatment with progesterone. The data are thus not as concise as one might desire, but they do show, in contrast to the claims of Smith and Albert (2) and of earlier reports (1, 3), that a definite decrease in gonadotropin excretion occurs during the course of treatment with progesterone.

METHODS AND MATERIALS

Eight patients, ranging in age from 30 to 64 years, were studied. In all but 1, the diagnosis of carcinoma of the cervix had been well established before treatment with progesterone was started. The latter course was decided on because each of the patients had experienced regrowth of the tumor and had already been treated to tolerance with radiation. Some of the patients were treated with progesterone by continuous intravenous infusion for nine to eleven days, and some by intravenous infusion for twelve hours each day for ten consecutive days. Doses ranged, on the average, from 100 to 400 mg. per day (Table 1). Six of the eight patients were menopausal by symptoms and history; 2, aged 30 and 41, had no symptoms of the menopause. Of the 6 menopausal patients, 2 had gone through a natural menopause fifteen and twenty-two years, respectively, before being included in this study; 3 had had a surgical menopause three, five and six years previously, respectively, and 1 had had menopausal symptoms since x-ray treatment six months before initiation of progesterone treatment (Table 1).

Progesterone for intravenous administration was dissolved in propylene glycol and human serum albumin according to a previously described method (20).

Urine was collected from each patient for twenty-four or forty-eight hours prior to starting progesterone treatment, and almost daily throughout the course of treatment. However, because of other studies which were being carried out simultaneously, only some of the urines were assayed for gonadotropins. Whenever possible the assays were directed toward determination of minimal and maximal levels.

Immature female mice of the Webster-Swiss strain were used; they were 21 days of age at the time of starting the assay. The urines were concentrated by the method of Gorbman (21), using an ultrafiltration apparatus, and the final residue suspended in the number of cubic centimeters of physiologic saline corresponding to the number of hours of excretion, so that each cubic centimeter of saline contained the equivalent of one hour's excretion of urine. Each mouse was injected with 0.5 cc. of the undiluted or diluted extract twice per day for three days and was killed by decapitation on the morning of the fourth day, at which time the uteri and ovaries were removed and weighed. A unit of gonadotropin was defined as that contained in the amount of urine (in terms of excretory hours) which would produce a doubling in weight of the uterus without an increase in the size of the ovaries.

RESULTS

The results are shown in Table 1. The first 6 patients listed in the table were menopausal by history and symptoms, and all but ER had levels of urinary gonadotropin within the expected range for menopausal subjects.

TABLE 1. EFFECT OF PROGESTERONE ON THE EXCRETION OF THE TOTAL GONADOTROPIN COMPLEX IN 8 PATIENTS WITH CARCINOMA OF THE CERVIX

Patient† & age	Progesterone therapy		Gonadotropin excretion (mouse units*/day)													
			Before progesterone		During progesterone therapy										After progesterone		
	Daily dose (mg.)	No. of days treated‡	Days		Days										Weeks		
			2	1	1	2	3	4	5	6	7	8	9	10	1	2	3
CL, 64	100	10(c)	—	48–96	<24	<24	< 9	< 9	< 9	—	> 9	> 9	< 9	—	> 8	<24	<24
GJ, 61	250	12(c)	12–24	48–96	12–24	—	—	—	—	—	—	—	—	48–96	48	24–48	—
HR, 42	220	9(c)	>96	24–48	>48	< 8	—	—	—	—	< 8	—	48–96	—	—	24–48	24–48
SS, 40	250	10(i)	48–240	—	24–48	—	8	—	—	—	—	—	—	<8	—	—	—
LT, 42	300	10(i)	—	>240	—	<24	—	24–48	<24	<24	—	<12	<12	12	>24	—	—
ER, 47	250	10(i)	8–10	8–24	—	< 8	—	—	—	< 8	—	—	—	48	—	—	—
VR, 41	270	10(c)	24–48	—	<12	<12	<12	<12	—	—	—	—	—	—	—	—	—
LG, 30	400	10(i)	>12	>16	>12	12	16	12–24	<12	<12	<12	—	<12	—	>12	< 9	> 9

* The figures shown in these columns are the mouse units per 24 hours. When two values are connected by a hyphen, the reader is to understand that the units found were greater than the first, but less than the second value. When neither a > nor a < sign is prefixed, the value is to be taken as the actual unitage found for that particular day.

† To conserve space in the table, the menstrual histories are given here. Patients CL and GJ underwent natural menopauses, 22 and 15 years, respectively, before this study. In Patients HR, SS and LT, the menopause followed surgery 5, 3 and 6 years, respectively, before this study. Patient ER had been menopausal for 6 months following irradiation. VR and LG were still menstruating regularly at the time the study was begun.

‡ (c) refers to continuous, or 24-hour per day infusion of progesterone.
(i) refers to intermittent, or 12-hour per day infusion of progesterone.

The last 2 patients were having menstrual cycles up to the time the study was begun. One of these women had a comparatively high level of urinary gonadotropin before progesterone was given, whereas the other had a level consistent with the non-menopausal state.

In 5 of the 6 menopausal patients there was a drop in the level of urinary gonadotropin during the course of progesterone treatment. In 3 cases (CL, SS and LT) there was a marked difference between the pre-progesterone level and that seen during the course of progesterone treatment. In 2 (HR and ER), the difference, though present, was not so marked. In Patient GJ there was no change in the level of gonadotropin excreted in response to progesterone treatment. There was a suggestive, but not clear-cut drop in gonadotropin excretion during the course of progesterone treatment in the 2 patients who were non-menopausal (VR and LG). During the post-progesterone period, there appeared to be a tendency for the excretion of gonadotropin to rise, and in 2 patients (HR and ER) this rise occurred toward the end of the period of progesterone therapy.

DISCUSSION

These data would suggest that progesterone in doses of 100 to 400 mg. per day given by the intravenous route, either as continuous or intermittent infusion, resulted in a decrease in the amount of total gonadotropin complex excreted into the urine. This was seen most clearly in the menopausal subjects in whom the initial excretion values were high, but some evidence for it was also seen in the 2 non-menopausal subjects. Admittedly, the data would be much less equivocal if more frequent assays could have been performed before, during and after the period of progesterone administration, or if control solutions, without progesterone, could have been infused. Due to the circumstances under which this study was carried out, neither of these things could be accomplished. However, if the data are looked at from the standpoint of the effectiveness of progesterone in inhibiting pituitary gonadotropin excretion in comparison with the well known effectiveness of the estrogens, they demonstrate an important point, namely, that progesterone is apparently effective in this respect, but is much less so than any of the estrogens. This is all the more remarkable in view of the undoubted effects of progesterone upon ovulation and the estrous cycle (5–18).

We do not believe that the study of Smith and Albert (2), which showed *no* effect of "progesterone" on urinary gonadotropin, vitiates this conclusion. They studied 2 patients; 1 was given 50.0 mg. of progesterone by mouth (a notoriously ineffective route of administration as far as biologic effect is concerned) and 1 was given 1.0, 10.0 or 100.0 mg. of anhydrohydroxyprogesterone (Pranone®) in three courses of a week each respec-

tively, with a three-week control period between each course of treatment. This steroid, however, is *not* progesterone, and any conclusions derived from its use cannot automatically be applied to the activity of progesterone.

The study of Büttner and Trappmann (3), which also showed a lack of effect of progesterone in inhibiting gonadotropin excretion, can be judged in the light of the findings presented here; namely, the doses used were very probably too small. They also studied 2 patients; 1 received a total dose of 60.0 mg. over a period of six days, and 1 received a total dose of 120.0 mg. over a period of eight days. As we have shown here, progesterone in a dosage of 100 to 400 mg. per day, although it apparently led to some decrease in gonadotropin excretion, *did not do so in every case.* Moreover, in the cases in which a decrease occurred, it was not marked.

The only valid conclusion we may draw at this point is that progesterone in large enough doses may induce a decrease in the excretion of the urinary gonadotropin complex, but it is far less effective in this respect than any of the estrogens.

SUMMARY

Eight female patients, in 7 of whom there was a proven diagnosis of carcinoma of the cervix, were treated with progesterone by the intravenous route for periods of nine to eleven days in dosages ranging from 100 to 400 mg. per day; the effect of this treatment on the excretion of the total gonadotropin complex was studied. In 5 of the 6 patients who were clearly menopausal, a definite decrease in the level of gonadotropin excretion was observed during the period of progesterone administration; in 1 there was no clear-cut change in relation to treatment. In the 2 non-menopausal patients there were possible, though not unmistakable, decreases in gonadotropin excretion during the period of administration.

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ADRENOCORTICAL FUNCTION IN ANXIOUS HUMAN SUBJECTS: THE DISAPPEARANCE OF HYDROCORTISONE FROM PLASMA AND ITS METABOLIC FATE*

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IN ANXIOUS human subjects the plasma level of hydrocortisone and the urinary output of a group of its metabolic products, the hydroxycorticoids, are almost twice those of normal controls (1). It is known that the quantity of hydroxycorticoids excreted by normal men represents about a fourth of the hydrocortisone produced by the adrenal cortex (2). The increased levels in the plasma and urine of anxious subjects may be due to an increased rate of hormonal production, or to a decreased rate of its disposal from plasma coupled with a metabolic shift in favor of greater hydroxycorticoid production from hydrocortisone. In order to interpret our findings, both aspects of the metabolism of hydrocortisone were investigated. This report concerns the rate of disposal of the hormone following the administration of a test dose of hydrocortisone and the proportion of that dose excreted as hydroxycorticoids in anxious and normal subjects.

SUBJECTS AND METHODS

Nine anxious patients (4 men, 5 women) hospitalized on a psychiatric service of a general hospital and 12 normal persons (5 men, 7 women) employed in the same hospital were the subjects of this study. All were volunteers. The anxious patients were selected by a psychiatrist from all the patients on the psychiatric service. They differed widely with regard to diagnosis but had in common some degree of anxiety or anxiety-proneness. They were able to communicate to the psychiatrist a feeling of dread or foreboding—as if something dangerous were impending. Such a degree of emotional arousal does not persist for a long period of time in a hospital setting. Consequently, most of this group consisted of patients who did not experience large amounts of anxiety over long periods of time, but were judged to be most prone to become anxious among our

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patient population. This appraisal was based on a past history of difficulty in coping with stressful life experiences (especially recent ones) and evidence of past weakness in psychologic defenses against anxiety (1, 3). The mean age of the 9 anxious patients was 37 years, and that of the controls was 32 years. All subjects were in good physical health and were not receiving during the course of this study any medication which could interfere with the chemical determination of hydrocortisone or hydroxycorticoids.

Several days prior to the administration of the test dose of hydrocortisone (pre-experimental day), a 24-hour urine sample was collected from each subject, in order to determine the excretion of endogenous hydroxycorticoids. On the experimental day, each subject was weighed and a blood sample was drawn for determination of the pre-experimental level of plasma hydrocortisone. Immediately thereafter the test dose of hydrocortisone was administered by intravenous infusion over a thirty-minute period. A dose of 1 mg. of hydrocortisone per kilogram of body weight was given to each subject. (Twenty milligrams of hydrocortisone were dissolved in each 100 cubic centimeters of 5 per cent glucose in water solution, as described by Brown (4) and his coworkers.) Blood samples were drawn from each subject at one, two, four and six hours after the beginning of the infusion for the determination of the levels of hydrocortisone in the plasma. A 24-hour urine sample was collected from each subject during the experimental day.

Plasma hydrocortisone was determined by the method of Nelson and Samuels (5, 6). Urinary hydroxycorticoids were assessed according to the method of Reddy, Jenkins and Thorn (7) as modified by Brown (4). Each pair of urine samples was checked for accuracy of collection by determination of the creatinine content (8).

RESULTS

Rate of removal of hydrocortisone from plasma

The level of endogenous hydrocortisone in the plasma of the anxious patients was 80 per cent greater than that of the normal subjects, resembling the findings in a larger population of anxious subjects from which the present subjects were drawn (1) (Fig. 1). Following the administration of hydrocortisone, the rate of disappearance of the steroid from the plasma

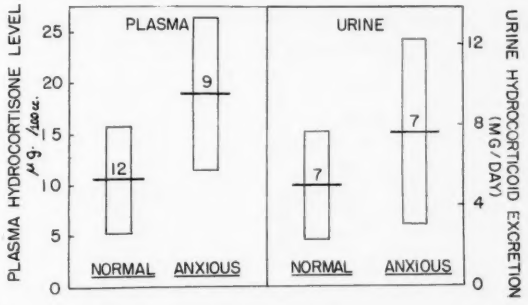

FIG. 1. The plasma level of hydrocortisone and the urinary excretion of hydroxycorticoids of anxious and normal subjects. The heavy black line is the mean of the group, the length of the bar represents ± one standard deviation and the number refers to the number of subjects in the group.

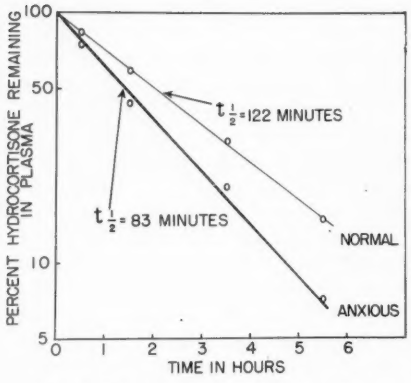

FIG. 2. The disappearance of hydrocortisone from the plasma of anxious and normal subjects following the administration of a test load of hydrocortisone.

may be obtained for each group from the plot of the hormone levels on a semilogarithmic scale against time. The rate of disappearance of hydrocortisone from plasma is expressed as a half-time, *i.e.*, the time at which the concentration achieved immediately following the administration of the test dose is reduced by half. The half-time of hydrocortisone disappearance for the normal group was almost identical to the values reported by other investigators (4, 9). The half-time in anxious patients was 82.7 minutes with a standard deviation of 21.8, whereas in the normal subjects it took 122.1 $\pm$ 20.5 minutes for the initial concentrations to become similarly reduced (Fig. 2). The difference in speed of clearance between the two groups as reflected in the half-times was significant at better than the 1 per cent level of confidence. In 8 of the 9 anxious patients hydrocortisone was removed faster from plasma than in any of the normal subjects.

When the line representing the rate of disappearance of hydrocortisone from plasma is extrapolated back to zero time, the concentration of hydrocortisone then in the plasma may be calculated. Presumably, this concentration occurs after complete mixing of the administered material and prior to the beginning of its metabolic destruction and/or removal. The zero-time concentration and its variability were almost identical for both groups of subjects, suggesting that the body compartment distribution of hydrocortisone did not differ in the two groups (Fig. 3).

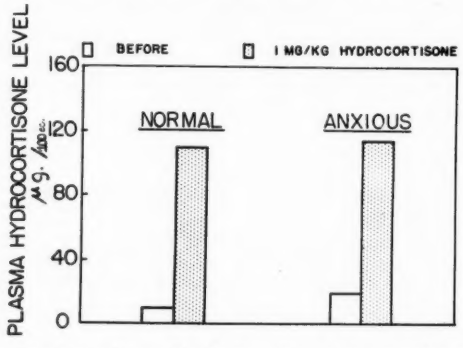

FIG. 3. The plasma hydrocortisone level of anxious and normal subjects before and immediately after the administration of a test load of hydrocortisone.

TABLE 1. URINARY EXCRETION OF HYDROXYCORTICOIDS AFTER HYDROCORTISONE INFUSION

Group	No. of subjects	Dose of hydrocortisone† (mg.)	Urinary hydroxycorticoids* (mg./day)			% Dose excreted
			Control day	Hydrocortisone day	Increment	
Normal subjects	7	68.3±11.2	4.96±2.65	21.77±8.78	16.81±7.52	23.0±9.0
Anxious patients	7	65.9±11.6	7.56±4.63	16.74±4.74	9.17±4.70	13.8±7.0
	t		1.29	1.33	2.28	2.12
	p		Not signif.	Not signif.	<0.05	0.05

* Complete urine collections obtained only on these subjects.
† Figures in the table are means ± standard deviations.

Excretion of hydroxycorticoids

On the pre-experimental day, the anxious subjects excreted 52 per cent more hydroxycorticoids than did the normal controls but the difference did not achieve statistical significance due to the large variability in the anxious group (Fig. 1; Table 1). The larger group of anxious patients from which this sample was drawn excreted almost twice the normal amount of hydroxycorticoids and the difference was significant (1). The difference in values between the anxious and normal groups of the present study is not due to variations in urine volume, since these were almost identical (anxious, 1338 ± 505 cc.; normal, 1440 ± 655 cc.). Following hydrocortisone administration, the hydroxycorticoid output of the normal subjects rose to a value 30 per cent greater than that of the anxious subjects. The increase in hydroxycorticoid excretion above the endogenous level for the normal group was 84 per cent greater than for the anxious group and the difference in increment was statistically significant. The normal subjects were presumably excreting a greater proportion of the ingested hydrocortisone as hydroxycorticoids than were the anxious subjects. The proportion of the dose excreted by the normal subjects compared favorably with the values reported in the literature from other laboratories (4, 10). Not one of the anxious patients excreted hydroxycorticoids in a proportion of the test dose that was greater than the mean for the normal group. The diminution in the proportion of hydrocortisone excreted as hydroxycorticoids by the anxious group indicates that these patients were metabolizing hydrocortisone differently than the controls, as well as removing it faster from the plasma. It is not known whether this different metabolic pathway involves a quicker destruction of hydrocortisone than ring A reduction and conjugation.

DISCUSSION

In the present study, three findings are reported which distinguish anxious from normal subjects. The anxious patients removed hydrocortisone

from plasma at a faster rate, excreted a smaller amount of hydroxycorticoids following a test load of hydrocortisone, and converted a smaller proportion of the administered hydrocortisone to hydroxycorticoids (tetrahydrocortisone+tetrahydrohydrocortisone).

The increased rate of removal of hydrocortisone from plasma in anxious subjects may be due to any of a variety of factors (such as faster conversion to tetrahydrocortisone, or faster conjugation), none of which have been experimentally tested. The failure of the anxious patients to excrete more hydroxycorticoids following a test dose of hydrocortisone than normal subjects suggests that the patients were metabolizing the ingested material differently. The present study does not provide any information as to what these metabolic products might be. However, it has been shown by others (11, 12, 13) that under severe psychologic stress giving rise to anxiety normal persons excrete greater quantities of 17-ketosteroids as well as of 17-hydroxycorticoids. In order to confirm the hypothesis that a shift in the metabolic disposal of hydrocortisone occurs in anxious subjects relative to normal controls, a balance study is essential. Such a project is presently under way, employing hydrocortisone-4-C^{14}.

This investigation was undertaken to account for the elevated blood and urine levels of hydrocortisone and hydroxycorticoids found in anxious patients. It had originally been deduced that these findings resulted from a decreased plasma clearance of hydrocortisone coupled with a shift in hydrocortisone metabolism to yield greater quantities of dihydroxy-acetone side-chain compounds. Actually, the situation was the exact opposite—increased plasma clearance coupled with a metabolic shift to yield lesser quantities of tetrahydrocortisone-like substances. Since these results were obtained when an exogenous load of hydrocortisone was imposed, the implication remains that if the subjects behave in a similar fashion with respect to the metabolism of their endogenous hormone production, their adrenal glands are producing hydrocortisone at an extremely rapid rate compared to normal controls. Despite a rate of disposal of hydrocortisone almost 50 per cent greater than that of normal subjects, the anxious patients maintained an endogenous blood and urine level almost double the normal value. Minimally, this would represent a rate of hormone production several times greater than that of a normal group.

SUMMARY

In anxious subjects hydrocortisone is removed faster from plasma than in normal controls. Despite a greater excretion of endogenous hydroxycorticoids by anxious patients, their excretion of hydroxycorticoids after the administration of a test load of hydrocortisone is less than in normal subjects. The proportion of the test load of hydrocortisone excreted as dihy-

droxy-acetone side-chain compounds by the anxious group is about half that in the normal group. It is concluded that anxious patients produce hydrocortisone at a much faster rate than normal subjects.

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ENDOCRINOLOGY OF ANOREXIA NERVOSA*

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THE physicians who earliest described anorexia nervosa considered it it to be of psychologic origin. Morton in 1689 (1), Naudeau in 1789 (2), Gull in 1874 (3), and Lasèque in 1873 (4) attributed the cachexia to emotional disturbances and this conclusion has been reaffirmed by the great majority of subsequent investigators.

However, the existence of an endocrinologic deficiency in these patients has been periodically suggested. This has been prompted by the presence of amenorrhea and a low basal metabolic rate. Simmonds' description of panhypopituitarism contributed to this opinion, since it inadvertently fostered the misconception that cachexia is a cardinal feature of hypophyseal failure and led to confusion of this disease with anorexia nervosa. Furthermore, some authors have been averse to a psychologic explanation of such a profound physiologic catastrophe. More recently certain metabolic and histologic abnormalities have been described which have been interpreted as further evidence of endocrinologic insufficiency.

There have been two different interpretations of the presumed glandular deficiency. Despite meager evidence, several authors have claimed that an endocrinologic disorder precedes the anorexia and is the etiologic factor. Ovarian (5, 6), thyroid (7) and pituitary insufficiency (8–12) have all been indicted as necessary antecedents. However, these theories have gained little general support. In contrast, other authors (13–16), although convinced of the *primary* role of a psychologic disturbance, have maintained that the ensuing malnutrition may itself cause hypophyseal failure which will lead to gonadal, thyroidal or adrenocortical insufficiency.

If the severe malnutrition of anorexia nervosa does produce functional panhypopituitarism, then the clinical, laboratory and pathologic picture should resemble that seen after subtotal or complete destruction of the anterior pituitary gland. This would be anticipated since there would be, in both conditions, an insufficiency of the various hypophyseal tropic hor-

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mones, leading to a failure of the target glands—the gonads, thyroid and adrenal cortex.

To assess this possibility the sequence of glandular failure following pituitary insufficiency due to destructive lesions must be considered as a model with which anorexia nervosa may be compared. When the pituitary becomes gradually incompetent, its "target" glands fail in a specific sequence (17), although there are rare exceptions (18, 19). Gonadal function and, probably, growth are affected earliest. Later (and this varies from weeks to years depending upon the amount and rate of hypophyseal destruction), thyroidal insufficiency appears, to be followed even later by evidence of adrenocortical failure. Apparently the gonadotropins are most vulnerable, the thyrotropins less so, and the adrenocorticotropins least of the three (17, 20). Excellent reviews of the subject are available by Escamilla and Lisser (21), Farquharson (22), Friedgood (23), and Perkins and Rynearson (24). Sheehan and Summers (25) have described in detail the atrophic and degenerative changes that occur in the ovaries, thyroid and adrenal, secondary to hypophyseal failure.

In an effort to clarify this problem, endocrinologic studies were carried out in 5 patients with anorexia nervosa. Three were severely cachectic; the other 2, though seriously malnourished, were not as emaciated. These subjects were selected, since it seemed probable that if anorexia nervosa were associated with glandular insufficiency, evidence of an abnormality would be present in such extreme cases. Detailed histories of these patients will be reported in a subsequent monograph (26).

METHODS

The 17-hydroxycorticosteroids (17,21-dihydroxy-20-ketosteroids) in the plasma were determined by the method of Nelson and Samuels (27). The concentrations in the urine of the 17-hydroxycorticosteroids and 17-ketosteroids after glucuronidase hydrolysis were measured by the method of Glenn and Nelson (28). The total urinary 17-ketosteroids were determined by the technique of Callow, *et al.* (29). The procedure of Migeon and Gardner (30), using fluorescence as described by Jailer (31), was employed to measure estrogens in the urine. The level of serum protein-bound iodine was determined by the method of Barker *et al.* (32), of butanol-extractable iodine by the method of Man *et al.* (33), and of corticotropin (ACTH) by the technique of Sydnor and Sayers (34). The urinary gonadotropins were determined in 1 patient (A.P.) by the alcohol concentration procedure of Klinefelter *et al.* (35), and in the other 2 patients (F.M. and E.M.) by Salhanick's modification (36) of the method of Bradbury *et al.* (37). Thyroidal radioactive iodine uptake was studied by the conventional techniques.

OBSERVATIONS AND DISCUSSION

Pituitary-gonadal function

In 2 of our cases, the concentration of gonadotropins in the urine was abnormally low, whereas in the third the value was in the low normal range

(Table 1). Data in the literature on this point have been inconsistent. Values have ranged from unusually low to high levels, although low titers have been reported more frequently. These inconsistencies are probably due to the limitations of the biologic assays for gonadotropin, since the techniques have been and still are, imperfect. However, our findings would suggest a gonadotropic deficiency, since the gonadotropins were abnormally low in 2 patients, and in the third a low normal value was associated with very scanty menstruation, suggesting a partial but not complete deficiency of pituitary "tropin."

Further confirmation of a gonadotropic insufficiency was the fact that abnormally small amounts of estrogens were excreted by the 2 subjects who were studied (Table 1). Data from the literature lend further weight to these observations, since a low excretion of estrogens has been the general finding, as well as an atrophic vaginal mucosa and a scanty, fibrotic or resting uterine endometrium (38–49).

If the ovarian failure were due to the direct effect of malnutrition upon the gonads, one would expect elevated excretion of gonadotropins, similar to that which occurs following surgical castration or the menopause. The absence of high values in our cases and in others reported in the literature, with the exception of several studied by McCullagh and Tupper (39), would favor the thesis of hypophyseal gonadotropic insufficiency as the cause of gonadal failure. Pathologic reports on cases of anorexia nervosa, although relatively few, would lend further credence to the notion of gonadal insufficiency (16, 39, 47, 50–64). The ovaries are usually small and atrophic. Occasional immature follicles may be found, but with rare exception, no graafian follicles, ova or corpora lutea are present. It would seem, therefore, that in anorexia nervosa there is partial or complete hypophyseal gonadotropic insufficiency, which leads to a gonadal failure. The identical situation is observed in malnourished experimental animals (65–68) as well as in patients with a true anterior pituitary failure.

Thyroidal function

The protein-bound iodine levels in the serum of 3 of our patients and the butanol-extractable iodine level in a fourth subject were normal (Table 1). Starr *et al.* (69) reported normal levels in 5 malnourished subjects and borderline values (3–4 μg.) in 4 others. However, these lower values must be understood in the context of the author's full report, since levels of 3 μg. or less were found in some cases of heart failure, senility, obesity, diabetes, neurosis and psychosis, in the absence of clinical evidence of hypothyroidism. Thyroidal I^{131} uptake at twenty-four hours was determined in 2 patients in our series; it was normal in both (Table 1). This conforms to the findings in other reports, since the I^{131} uptake has been normal in every

TABLE 1. ENDOCRINOLOGIC VALUES IN 5 CASES OF ANOREXIA NERVOSA

Subject	Urine vol. (ml./24 hrs.)	Urinary creatinine (mg./24 hrs.)	Urinary 17-KS (mg./24 hrs.)		Urinary 17-OH-CS (mg./24 hrs.)	Urinary estrogens (μg./24 hrs.)	Gonadotropins	Plasma 17-OH-CS (μg./100 ml.)	ACTH in blood, by adrenal ascorbic-acid depletion (mg./Gm. fresh adrenal)	Serum PBI (μg./100 ml.)	Serum butanol-extractable iodine (μg./100 ml.)	Thyroidal I^{131} uptake (% in 24 hrs.)
			Total	Glucur-onides								
A.P. ♀ wt. 60 lbs.	585	0.53	3.2	0.2	0.2		low to 0 units (abnormally low post-menopausally	21	11, −1, 17, −35 (average, −2)	4.0		
	428	0.49	2.2	0.2	0.2			20 (normal value)				
F.M. ♀ wt. 60 lbs.	1875	0.43	1.9	1.0	2.9	5.5	0 units	26		6.1		
	2225	0.49	1.3	0.7	2.6	5.0						
E.M. ♀ wt. 45 lbs.	2120	0.42			1.9		20 units (low normal value)	22		6.1		51
	3000	0.42	6.1	1.5	2.6	10.5						
	1412	0.27	1.9	0.4	0.9	3.0						
	2380	0.43			1.3							
D.H. ♀ wt 78 lbs.								17				
R.J. ♂ wt. 114 lbs.											3.5	30
Normal values		*1.0–2.0*	*5–15*	*2–11*	*2.1–5.8*	*20–50*		*3–34*		*4.0–8.0*	*3.2–7.2*	*10–50*

case cited in the literature, although several have been in the low-normal range (10–20 per cent) (16, 70, 71). A particularly interesting patient with anorexia nervosa was studied by Perloff *et al.* (16). Despite a ten-year history of severe cachexia finally terminating in death, her thyroidal I^{131} uptake at the end of this period, when she weighed 56 pounds, was 28 per cent. In contrast, patients with structural hypopituitarism, when thyroid insufficiency intervenes, have abnormally low levels of serum protein-bound iodine or its equivalent in the peripheral blood (17, 72, 73) as well as thyroidal I^{131} uptake values which are abnormally low or in the low-normal range (71–76).

Clinical observations in cases of anorexia nervosa lend credence to the absence of thyroid insufficiency. To our knowledge, from a diligent survey of the literature, no case has been reported with signs of myxedema. Furthermore, the characteristic asthenia, sluggishness and mental retardation of thyroid insufficiency is in direct contrast with the usual remarkable activity and mental alertness of the cachectic patient with anorexia nervosa. It is true that basal metabolic rates may range as low as −40 per cent in some of these patients (77), but this is due to a compensatory hypometabolism and not to a deficiency of thyroid hormone.

Changes observed in the thyroid gland at autopsy in cases of anorexia nervosa have varied. The gland has been reported either as normal or having follicles distended with thick colloid and lined by flat epithelial cells. In no case has extensive fibrosis, significant lymphoid infiltration or pervasive destruction been seen, as may occur in structural panhypopituitarism (16, 39, 47, 50–64). It would seem that in anorexia nervosa, the thyroid gland is functionally normal, since the results of specific tests of its activity are normal and histologic changes at autopsy are minimal.

Adrenocortical function

In 4 of our cases of anorexia nervosa, the titer of circulating adrenal steroids in the blood was in the high-normal range (78) (Table 1). Intravenous administration of corticotropin (ACTH) promoted a normally prompt increase in these levels (79) (Fig. 1). Such observations indicate an adrenal gland not only supplying the tissues with adequate concentrations of adrenal steroids, but one able to secrete additional hormone rapidly when stimulated. This is in contrast to cases of structural hypopituitarism, in which blood adrenal steroid levels are lower (80, 81) and the initial response to corticotropin is small (81–83). Furthermore, the level of circulating corticotropin, which was determined in 1 of our patients, was below measurable values. In cases of adrenocortical insufficiency significant levels have been found, whereas those in normal subjects were too low to be measured (84).

However, in agreement with the observations of most other investigators (85–90), the concentration of adrenal metabolic products in the urine tended to be low in the 4 patients who were studied (Table 1). Low values in cases of anorexia nervosa have been interpreted by many as evidence of adrenocortical insufficiency, but this is an unlikely explanation in view of the blood steroid observations. Although these low levels might be due to changes in renal function, a more likely answer, in the presence of such drastic malnutrition, would be the reduction in metabolism. The gland supplies adequate quantities of hormone: it responds normally to stimulation; but, as little hormone is probably being metabolized, less is necessary to maintain normal levels in the body fluids, and consequently smaller

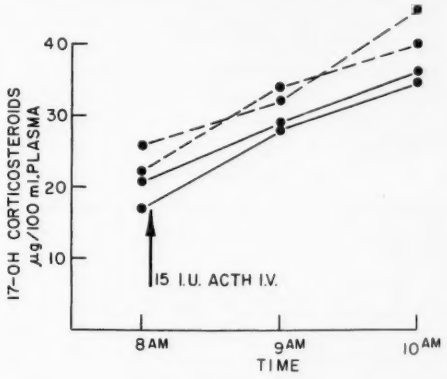

FIG. 1. Effect of intravenous corticotropin (ACTH) on the level of plasma 17-hydroxycorticoids in anorexia nervosa.

amounts of 17-hydroxycorticosteroids and 17-ketosteroids are excreted into the urine.

Normal findings with the Kepler-Power water test have been reported in these subjects (16, 38, 44) and except for 2 cases of Perloff *et al.* (16), the results of the Thorn ACTH test have also been normal (38, 44, 86, 87).

Minimal changes have been seen in the adrenal cortex at autopsy in most cases of anorexia nervosa (16, 39, 47, 50–64). Such alterations as loss of lipid or change in the size of the gland are nonspecific findings common to many pathologic conditions, and need not be indicative of glandular incompetence (91). These changes are not comparable to the atrophic and sclerotic transformations seen at times in cases of panhypopituitarism. However, in 2 autopsy cases of anorexia nervosa, sclerosis and partial obliteration of the zona glomerulosa were reported (51, 52, 62). It is difficult to reconcile these isolated observations with the negative findings in all other cases, many of which were as chronic and associated with as severe

emaciation. Clinically, adrenal crises may occur late in the course of true panhypopituitarism; they have not been reported in anorexia nervosa. It appears, therefore, from clinical, physiologic and pathologic observations that adrenocortical function is unimpaired in these subjects.

SUMMARY

Gonadal failure, probably due to gonadotropic insufficiency, appears to be the only endocrinologic disturbance in anorexia nervosa. There is no convincing evidence that the pituitary thyrotropic or adrenocorticotropic hormones are affected by the malnutrition; nor is there evidence that thyroid or adrenocortical insufficiency occurs.

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DETERMINATION OF TETRAHYDROCORTISOL AND TETRAHYDROCORTISONE IN THE URINE OF NORMAL AND SCHIZOPHRENIC MEN*

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IN THE course of determining human urinary excretion patterns by paper chromatography (1, 2, 3), tetrahydrocortisol (THF) or pregnanetetrol-3α,11β,17α-21-one-20 and tetrahydrocortisone (THE) or pregnanetriol-3α,17α,21-dione-11,20 were found to be present in every extract from a series of 37 subjects. Frequently aliquots of these two eluted steroids were taken and the quantities present estimated colorimetrically with the blue tetrazolium reaction (4). The preliminary determinations indicated a fairly consistent ratio of THE to THF. Meanwhile, Baggett, Kinsella and Doisy (5) had determined by paper chromatography and the Porter-Silber reaction (PS) (6) the amounts of THE present in urinary extracts from a series of 10 subjects. Accordingly, it appeared feasible to determine routinely the ratios of excretion of these two important urinary steroids, THE and THF, to each other and to the total 17,21-dihydroxy-20-ketosteroid (PS) output in a relatively small aliquot of a 24-hour urine sample. The results obtained for 40 urine samples collected under varying circumstances from an Air Force bomber crew of 8 men, and for 15 samples obtained from male schizophrenics from the Worcester State Hospital are presented.

METHOD

A two-hour aliquot of a urine sample was brought to a minimum volume of 100 cc. by addition of water, the pH adjusted to 6.5 and stabilized by addition of 10 cc. of phosphate buffer. The urine was incubated for twenty-four hours at 37° C. with bacterial glucuronidase (Sigma Chemical Co.) using a minimum of 35 Fishman units per cc. of adjusted urine volume.

After incubation the samples were extracted with redistilled ethyl acetate (2×60; 3×40 cc.). The ethyl acetate extracts were washed with cold 0.1 *N* NaOH (3×25 cc.) and with distilled water (3×25 cc.). A further extraction of the combined water washes was made with ethyl acetate (3×20 cc.) and added to the initial one. The extracts were then shaken with 1 Gm. of anhydrous Na_2SO_4 and transferred with rinsing to a 500-cc. round-bottom flask.

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The ethyl acetate extract was concentrated in the flask and placed in a warm water bath at 40° C., using vacuum distillation under a stream of nitrogen. When concentration to a 10–15 cc. volume was effected, the remaining ethyl acetate was evaporated under a stream of nitrogen at 40° C. at atmospheric pressure.

Appropriate aliquots were removed for a Porter-Silber determination (6) and the residue transferred to tubes suitable for subsequent transfer to paper chromatograms. The paper chromatography was carried out as previously described (1, 2) in the benzene-formamide system (7) at 30° C., using washed Whatman #42 paper, which was wet with a 1:1 methanol-formamide solution. Strip widths were determined by the PS value for the total extract, and varied from 1.0 to 2.0 cm. THE or THF was always applied to adjacent strips as comparison standards. After the strips were developed for seventy-two hours and dried, the THE and THF were located on 0.1-cm. strips cut from the chromatogram, using blue tetrazolium (BTZ) (4). The remaining areas of the paper containing the THE and THF were then cut up and eluted four times with a 1:1 methanol-chloroform solution which was evaporated off. Aliquots were taken for determination of the 17,21-dihydroxy-20-ketosteroid (PS) content.

The PS values were determined using the modified Silber-Porter method (6) with recrystallized phenylhydrazine. The color reaction was carried out either in a water bath at 60° C. for one hour, or at room temperature overnight. The volumes were adjusted to a total of 3 cc. for use in silica cells of 10-mm. light path $\times$ 48 mm. in the Beckman DU spectrophotometer. The readings were made at 410 mμ. All values are expressed as mg. per twenty-four hours and have been corrected for both the portion of the test strip cut off and a 20 per cent loss of material occurring as a result of paper chromatography. An additional correction has been made for the aliquots removed for the initial PS determination.

RESULTS

Preliminary Experiments

Efficiency of solvent extraction of PS-reacting material by handshaking of urine increased in the following order: ether-chloroform 1:3, methylene chloride, isopropyl acetate, ethyl acetate. The ethyl acetate along with the isopropyl acetate had the further advantage of extracting relatively less urinary pigment contributing to the optical density (O.D.) readings of the blanks in the PS reaction.

The efficiency of extraction of PS-reacting material with ethyl acetate was approximately 90 per cent. A mean increment of 11.0 per cent $\pm$ 0.57 (S.E.) over the initial PS value was obtained by re-extraction of 10 urine samples. Re-extraction of the water washes with ethyl acetate yielded increments of 0–2 per cent over the original value.

Recovery of THE added to duplicate urine samples was as follows: 25 μg., 80 per cent; 50 μg., 89 per cent; 250 μg., 104 per cent; and 500 μg., 108 per cent. Recovery of THF added to quadruplicate samples was: 50 μg., 90 per cent; and 250 μg., 98 per cent. THE was applied to duplicate paper chromatograms in amounts of 50, 100 and 200 μg., and to quadruplicate chromatograms in amounts of 400 μg. A mean recovery of 80 per cent was obtained. The mean recovery of THF applied to duplicate strips in 50, 100 and 200-μg. amounts was 79 per cent. (Concentration appeared to have no effect on recoverability, since 82 per cent of both 50-μg. and 400-μg. amounts of THE was recovered). The total mean recovery for both compounds was 79.4 per cent $\pm$ 1.30 (S.E.). As a result, a correction factor for a loss of 20 per cent was applied to the PS values obtained after paper chromatography.

In addition, each of 6 urinary extracts was applied to a paper chromatogram to determine the recovery of PS-reacting material. The paper chromatograms were cut into

5 portions corresponding to 1) the area more polar than THF, 2) THF, 3) THE, 4) the cortisol area, 5) the cortisone area. PS determinations were made on aliquots of eluates of these and of the run-off. Recoveries, using the correction factor, ranged from 76 to 116 per cent, with a mean recovery of 93 per cent.

Extractions of urine samples were made at both pH 7.0 and pH 1.0. Under the latter circumstances, there was an average increase of 20 per cent in the PS value. A series of urine samples was then extracted at pH 7.0, and half of the series was re-extracted at the the same pH. The other half was re-extracted at pH 1.0. A 10 per cent increase was obtained for the former—a 20 per cent increase over the PS value obtained for the latter highly pigmented extracts. Both extracts, after paper chromatography, contained trace amounts of the same order of BTZ-reacting metabolites with running rates similar to those of THE and THF. However a faintly BTZ-positive area of paper eluted from the pH-1.0 strip in the region between cortisol and cortisone yielded a PS value accounting for 40 per cent of the PS increment. An orange crystalline substance was subsequently isolated from an accumulation of this material; 10, 20 and 30 μg. yielded PS values equivalent to 3, 7 and 10 μg. respectively of THE. In view of the fact that no additional THE or THF was recovered at pH 1.0 and that the additional pigment interfered with both the PS reaction and the paper chromatography, extractions were carried out at pH 7.0.

The PS optical densities (O.D.) for both THE and THF were proportional to concentrations from 2 to 40 μg., with a mean O.D. of .210 for 10 μg. of THE, and .158 for 10 μg. of THF. Substitution of HCl for H_2SO_4 in the PS reagent proved unsatisfactory in our hands. The optical densities of PS urine blanks were found to increase proportionally with decreasing pH of urine extraction. After pH 1.0 extractions, urine blanks usually had an O.D. equivalent to two-thirds of the phenylhydrazine-sulfuric acid (PHSA) urine value. This was reduced to a quarter or a half at pH 7.0. After paper chromatography, the O.D. of the eluted THE and THF blank aliquots ranged from less than 1 per cent to 20 per cent of the PHSA value, with most values falling between 5 and 15 per cent. In no instance did an O.D. for the urine blank exceed that for its companion PHSA aliquot. The PS values for THF are expressed both in terms of THE and THF standard, since equivalent amounts of the latter absorbed approximately 75 per cent as much light as the former under the circumstances described.

Preliminary identifications of the urinary metabolites presumed to be pregnanetetrol 3α,11β,17α,21-one-20 and pregnanetriol-3α,17α,21-dione-11,20 were made by determination of sulfuric-acid chromogen spectra on aliquots from the eluted THF and THE spots. Typical spectra were obtained with maxima of 315, 410 and 510 mμ for the former and 335–340 and 410 mμ for the latter. In addition, the remainder of the THE-like and THF-like eluates from the paper chromatograms of the Air Force crew and the schizophrenics were pooled for purposes of isolation and further identification of these compounds. Crystalline THF, the infrared spectrum of which was identical with that of known pregnanetetrol-3α,11β,17α,21-one-20, was isolated from the THF eluate pool. However, a high-melting crystalline material obviously not THE was obtained from the THE eluate pool. This compound proved to have an infrared spectrum identical with that for Reichstein's compound C, allopregnanetetrol-3α,11β,17α,21-one-20 (allo-THF), which we have previously isolated from human urine as a radioactive metabolite after administration of C^{14}- and carrier-cortisol. (The data concerning this compound will appear elsewhere.) The mother liquor from this crystallization was subjected to further paper chromatography in the chloroform-formamide system (8), to separate any allo-THF from THE. The presumed THE was eluted and a crystalline compound, the infrared spectrum of which was the same as that for pregnanetriol-3α,17α,21-dione-11,20, was obtained from the eluate.

Results for Schizophrenics and the Air Force Crew

Two-hour aliquots of 24-hour urine samples collected from 15 male schizophrenics, aged 24 to 56, were analyzed as outlined. In the case of the 8 Air Force crew members, aged 24 to 44, two-hour aliquots were taken from 40 urine samples collected under the following circumstances: 1) 8 control samples from non-flying subjects (AM-DAY control), representing the first third of a 24-hour period consisting of collections throughout the morning, up to, and including midafternoon; 2) 8 control samples (DAY-PM control), representing the remainder of the 24-hour period from mid-afternoon through the night and including a collection on rising in the morning; 3) and 4) 16 samples (Day Flight), collected from noontime to late evening during a flying mission; and 5) 8 samples (PM Flight), collected during an all-night flying mission. By combining the PS values for the AM-DAY and DAY-PM control periods the PS output for twenty-four hours was obtained.

The mean excretion values for the total PS, THE and THF in mg. per twenty-four hours, the output ratios, and other related data for the 15 schizophrenics and 8 Air Force crewmen are presented in Table 1. The mean total PS values for 24-hour samples were 6.4 mg. for both the Air Force crewmen and schizophrenics—range, 2.3 to 12.0 mg. The PS value for THE, similar for both groups, was 3.8 mg. per twenty-four hours—range, 1.4 to 8.3 mg. In the case of the schizophrenics the THF value of 1.6 mg. per twenty-four hours expressed in THE equivalents was higher than that for the Air Force crew, 1.3 mg. The range was 0.7 to 4.2 mg. for the schizophrenics and 0.5 to 2.7 mg. for the AF crew. This non-significant difference is reflected in the slight shift of the mean ratio, THE:THF, to 71:29 per cent for the schizophrenics and 75:25 per cent for the AF crew. The small standard errors for these ratios indicate, however, a rather constant ratio of excretion of THE to THF from individual to individual. Approximately 80 per cent of the total PS extract was accounted for as THE and THF.

Owing to a discrepancy between the optical densities of equivalent amounts of THE and THF, the latter values are presented in Table 1 in equivalents of both standard THE and THF. When THF is expressed in terms of THF standard the actual ratio of excretion of THE to THF was approximately 2:1 instead of 3:1.

The data obtained for the Air Force crew during control periods and flight periods are compared in Table 2. The mean PS values of the control periods reflected a previously observed diurnal change in steroid excretion (9, 10), wherein morning values were considerably increased over those for the night. The mean flight values fell between those for the AM-DAY and DAY-PM control periods. The mean PS value for the total extract of the

TABLE 1. MEAN EXCRETION VALUES AND OUTPUT RATIOS OF TOTAL PS, THE AND THF (MG. PER 24 HRS.) FOR NORMAL AND SCHIZOPHRENIC MEN

	Air Force Crew (8 men)		Schizophrenics (15 men)	
	Mean ±S.E.	Range	Mean ±S.E.	Range
Age (yrs.)	32*	24–44	38*	24–56
Creatinine (Gm./24 hrs.)	1.79±0.16	1.4–2.6	1.69±0.17	0.9–2.8
	Values expressed in equivalents of THE			
Total PS (mg./24 hrs.)	6.42±0.91	4.1–12.0	6.44±0.59	2.3–10.0
Total PS (mg./Gm. creat. ×100)	3.57±0.31	2.5–4.6	3.96±0.25	1.9–5.2
THE+THF (mg./24 hrs.)	5.10±1.04	2.0–10.6	5.39±0.67	2.1–11.2
THE (mg./24 hrs.)	3.81±0.78	1.6–8.3	3.82±0.47	1.4–7.1
THF (mg./24 hrs.)	1.30±0.28	0.5–2.7	1.57±0.23	0.7–4.2
% THE of THE+THF	74.6±1.57	68–81	71.1±1.22	63–81
% THE+THF of total PS	75.5±5.69	50–104	84.7±5.91	43–134
	THF expressed in equivalents of THF			
THE+THF (mg./24 hrs.)	5.54±1.11	2.2–11.4	5.89±0.74	2.3–12.6
THE (mg./24 hrs.)	3.81±0.78	1.6–8.3	3.82±0.47	1.4–7.1
THF (mg./24 hrs.)	1.74±0.37	0.6–3.5	2.07±0.37	0.9–5.6
% THE of THE+THF	68.8±1.67	61–76	65.0±1.49	56–79

* Median age.

DAY-PM control was significantly less than that for the AM-DAY control ($t=2.20$ at the 5 per cent level of confidence).

By calculating output changes, using each subject as his own control, a pronounced diurnal rhythm was indicated for total PS, THE and THF by a highly significant reduction in their excretion during the DAY-PM control period as compared with the AM-DAY control period. The percentile decreases in output were as follows: total PS—61.5 per cent ±S.E. 6.08, $t=6.08$; THE—66.6 per cent ±7.47, $t=4.45$; THF—57.3 per cent ±7.53, $t=5.68$; and THE±THF—62.8 per cent ±5.06, $t=6.17$. All these values are significantly different at the 0.1 per cent level of confidence.

The mean ratios of excretion of THE and THF in terms of percentage of THE compared to THE+THF (mg. per twenty-four hours) are presented for control and flight periods in Table 3. Regardless of changes in absolute output of THE and THF as contrasted in the AM-DAY and PM control

TABLE 2. MEAN EXCRETION AND RANGE OF VALUES OF TOTAL PS, THE AND THF (MG. PER 24 HRS.) FOR AIR FORCE CREW MEMBERS DURING CONTROL AND FLIGHT PERIODS

Period	Time of day	Type of sample	Number	PS—mean values (mg./24 hrs.)*				THE+THF, as % of total extract
				Total extract	THE+THF	THE	THF	
1	AM-DAY	Control	8	8.84± 1.75† 4.7 – 20.2	6.88± 1.65 2.5 – 16.9	4.98± 1.25 1.9 – 12.9	1.95±0.53 0.5 – 4.6	70.4±5.55
2	DAY-PM	Control	8	4.85± 0.46 3.2 – 6.7	3.92± 0.69 1.8 – 7.2	3.02± 0.58 1.4 – 5.8	0.88±0.12 0.4 – 1.4	76.1±6.30
3	DAY	Flight	8	6.59± 0.94 3.5 – 12.3	5.57± 1.39 2.6 – 14.3	4.28± 1.18 1.6 – 11.6	1.29±0.23 0.7 – 2.8	78.3±7.29
4	DAY	Flight	8	6.51± 1.13 3.2 – 12.5	5.33± 1.41 2.2 – 14.3	4.10± 1.22 1.6 – 12.0	1.23±0.24 0.6 – 2.3	75.9±9.03
5	PM	Flight	8	6.37± 0.72 4.1 – 9.2	4.48± 0.57 2.3 – 6.8	3.46± 0.47 1.7 – 5.6	1.10±0.22 0.7 – 2.6	69.6±3.40
			40					74.1±3.03

* Expressed in THE equivalents.
† Standard Error.

values, the ratio of excretion of these two compounds was remarkably constant. The mean ratios were altered neither by effects of the diurnal rhythm nor by flight. Furthermore, the small standard errors indicate that the ratios varied little both within and between individuals.

DISCUSSION

The data of this paper demonstrate: (a) a convenient method for the separation and quantitative measurement of tetrahydrocortisol (THF)

TABLE 3. MEAN RATIOS OF EXCRETION OF THE AND THF FOR AIR FORCE CREW MEMBERS DURING CONTROL AND FLIGHT PERIODS

Period	Time of day	Type of sample	Number	% THE (mg./24 hrs.)* / THE & THF (mg./24 hrs.)
1	AM-DAY	Control	8	75.0±1.69†
2	DAY-PM	Control	8	76.1±1.32
3	DAY	Flight	8	73.9±2.63
4	DAY	Flight	8	74.7±2.65
5	PM	Flight	8	75.4±2.66
			40	75.0±1.01

* Expressed in THE equivalents.
† Standard Error.

and tetrahydrocortisone (THE), the principal metabolites of cortisol and cortisone in human urinary extracts; (b) that THE may be contaminated with unknown amounts of allo-THF; (c) that these two metabolites, as measured, constitute 75–80 per cent of the extractable 17-hydroxysteroids measured as Porter-Silber (PS) chromogen; (d) that the absolute amounts of total PS, THF and THE excreted did not differ significantly in extracts from urines of the normal and schizophrenic men studied; (e) that Air Force crewmen exhibited a diurnal rhythm in the urinary excretion of total PS, THF and THE; (f) that the ratio of THE:THF was remarkably constant in both the Air Force crewmen and the schizophrenics, and remained constant despite variations in absolute output rates; and (g) that this ratio was somewhat lower in the urine of the schizophrenic men, but not significantly so.

Several of these findings require further comment. Thus, Baggett *et al.* (5) reported an output of THE ranging from 2.0 to 7.4 mg. per twenty-four hours, a range overlapped by our values of 1.6 to 8.3 mg. Only Gold, Macfarlane and Moore (11) have measured the quantities of THF, as the formazan derivative, in relation to effects of corticotropin and surgical stress in 2 subjects. Furthermore if allo-THF is a regular urinary constituent, the contribution of 11β-hydroxy derivatives of cortisol and cortisone to the urinary pool of metabolites may be considerably higher than heretofore thought. We are at present investigating the possible quantitative significance of allo-THF in a series of urines from human subjects.

These data demonstrate no difference in the output of PS chromogen between schizophrenic men and Air Force crewmen. In addition, PS chromogens were determined on half-hour aliquots of 24-hour urine samples from a group of 25 normal men, aged 24 to 49 (median age, 37). These values ranged from 3.5 to 12.2 mg. per twenty-four hours, with a mean of 6.4 ± 0.41 mg. This mean value does not differ significantly from that for the schizophrenic subjects. Furthermore the PS values observed are in a range similar to that recorded in the literature (12–17) for normal men.

We previously reported (18, 19) a significantly subnormal excretion of neutral reducing lipids during short-term "pre-stress" periods in groups of younger schizophrenic patients, but this did not occur in older schizophrenics (20). Since the schizophrenic subjects of the present study are comparable to the previously studied younger schizophrenics, it follows that either: (a) the 24-hour urine collection smooths out differences detectable in short-term collections, due perhaps to a difference in diurnal rhythm compared to that of normal subjects, or (b) substances contributing to the value for reducing lipids are excreted in reduced amount by schizophrenic subjects, or (c) enzymatic hydrolysis and ethyl acetate extraction have provided a more efficient method as compared to extraction

at pH 1.0 and determination of the rather nonspecific reducing lipid as previously employed. In any case, we are in the process of further elucidation of this situation.

The remarkable constancy of the THE:THF ratio which we have observed occurs in the face of diurnal rhythm changes, the stress of flying, and in the schizophrenic state. If we consider that this ratio must represent an equilibrium between the two major metabolites of cortisol secreted by the adrenal cortex it follows that a rather stringently regulated transformation or excretion process, or both, must exist. Conceivably this ratio may be regulated at the kidney level, but it seems more likely that equilibrium is maintained in the tissues. Accordingly, an examination of this ratio in various diseases may be worth undertaking if only to discover those pathologic conditions in which it is upset. Since 11-ketocorticosteroids are readily converted to 11-hydroxycorticosteroids and vice versa (21), this equilibrium may represent a constant of some biologic significance.

SUMMARY

A method has been presented for the routine estimation in urinary extracts of THF (tetrahydrocortisol or pregnanetetrol-3α,11β,17α,21-one-20) and THE (tetrahydrocortisone or pregnanetriol-3α,17α,21-dione-11,20) $\pm$allopregnanetetrol-3α,11β,17α,21-one-20, which constitute approximately 75–80 per cent of the total 17,21-dihydroxy-20-ketosteroids excreted. The output of these two metabolites by normal and schizophrenic men did not differ substantially in twenty-four hours. The expected diurnal rhythm in the excretion of THE and THF was confirmed. The ratio of excretion of THE to THF (approximately 2:1) appears to be remarkably constant in men.

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DETERMINATION OF BASAL RATE OF OXYGEN CONSUMPTION BY OPEN AND CLOSED-CIRCUIT METHODS

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INTRODUCTION

FOR many years the routine clinical measurement of basal metabolic rate (BMR) at this institution has been performed with the open-circuit method, as used to establish the Mayo Foundation Standards (1). However, newer laboratory methods for evaluating thyroid function, such as measurement of the protein-bound iodine concentration in serum and measurements utilizing radioiodine, now provide additional information. In evaluating the relative utility and complexity of the various procedures, the question arose as to the degree of accuracy provided by the "simpler" closed-circuit method for measuring oxygen consumption that is in almost universal clinical usage. The limitations of available data, as noted later in this paper, made it advisable to compare the results obtained by both open and closed-circuit methods in a series of patients having routine tests. In some cases the errors of the closed-circuit method were of clinical importance. The sources of these errors, including some that have not been generally recognized, are discussed. This presentation is concerned with the measurement of the rate of oxygen consumption, and not with the question of the metabolic standards with which it is compared (2).

MATERIAL AND METHODS

Measurements were made on 52 patients who were having routine tests, and who were taken at random, excluding only persons who had neither teeth nor dentures. The group included 37 women between 19 and 74 years of age (average, 47 years) and 15 men between 19 and 71 years of age (average, 48 years). The series was terminated when the results noted later in this paper, including the magnitude and frequency of deviations between open and closed-circuit methods, became sufficient for present purposes.

Tests were conducted between 7:30 and 10:00 A.M., following an overnight fast. After the patient had rested supine for twenty or more minutes, open-circuit measurements were conducted by technicians, essentially as described by Boothby and Sandiford (3).

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Briefly, the patient breathed room air through an oronasal mask. Expired air was collected for ten minutes in a Tissot spirometer and analyzed in duplicate with a Haldane apparatus.

After several minutes, closed-circuit recordings were obtained for nine to ten minutes, using mouthpiece, noseclip and a Benedict-Roth type spirometer. Previous work (4) and results later in this paper justified the absence of alternate order of tests. Initially, the spirometer was rinsed with air, and then about 3 liters of air and 3 liters of oxygen were added. Outboard leaks were checked during the first several minutes of the test by noting the apparent presence or absence of a change of slope of the recording after a weight had been placed on the bell of the spirometer. All closed-circuit recordings and their graphic analyses were made by one of us (Fowler). The graph for the first three to four minutes was not used in determining the slope of the recording. Both changes of volume and time were determined by measurement. A correction of 0.8 mm. was added to the observed change of position of the spirometer bell to compensate for the increase of temperature (0.5° C.) of gases within the spirometer during recording. Conversion of volume changes to caloric values was made in accordance with the manufacturer's instruction.[1] The volumetric calibration of the spirometer was established within 1 per cent by measurements of the circumference of the bell and by the weight of water displaced. The aqueous saturation of spirometer gas, about 80 per cent by dew point, was in accordance with the correction table of Wilson (5) provided with instructions. The soda lime was replaced after 35 tests, although at this time the carbon dioxide content of gas inspired from the spirometer during a determination was less than that of room air (0.04 per cent) as determined by a Liston-Becker infrared analyzer. Mayo Foundation Standards (1) were used to express caloric values as basal metabolic rate.

RESULTS

All open-circuit measurements were considered to be satisfactory, as judged by relaxation of the patient, by observation of pulse rate and character and frequency of respiration during the test period and their correspondence to observations made during the preliminary rest period, and by the respiratory quotient (R.Q.) calculated from analysis of expired air.

During closed-circuit tests, nasal expiratory leaks occurring in 3 patients could not be eliminated by several adjustments of the noseclip during a period of ten to twelve minutes. The apparent BMR of these tracings would have been 20 to 50 per cent too high. Results of the two methods were not compared for these 3 patients or for 1 other patient whose pulse rate was elevated during one of the tests. The results of 49 comparisons on 48 patients are shown in Figure 1. A correlation between values of calories per square meter per hour (cal./m.^{2}/hr.) was present but not close ($r = 0.84$). The best-fitting line of linear regression of cal./m.^{2}/hr. by closed circuit (Y) on the similar value by open circuit (X) was $Y = 2.04 + 0.94\ X$. The standard error of the regression coefficient was ± 0.087 and the standard error of estimate of Y from X was ± 2.63 cal./m.^{2}/hr.

Absence of a systematic difference is also shown in Table 1, which pre-

[1] Warren E. Collins, Inc., Boston, Massachusetts.

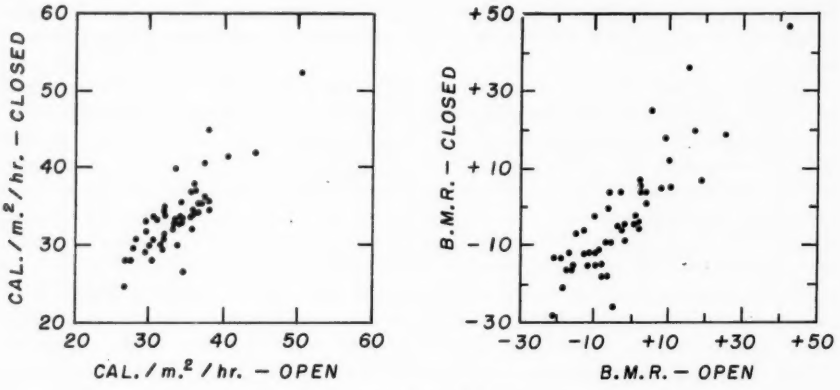

FIG. 1. Relation between values obtained by open-circuit and closed-circuit methods.

sents the deviations of values by closed-circuit technic from those by open-circuit technic. Because some variation was found between consecutive tests with the open-circuit methods, the values of the deviations which would remain after correction for an intra-daily variation of ±0.96 cal./m.²/hr. (6) are also listed. Separate calculations were made for 36 recordings that were qualitatively graded as "more regular" and are illustrated in Figure 2. Deviations were considerable even for the "more regular" recordings, and conversely some "less regular" recordings agreed closely with open-circuit values. Scattergraphs indicated that the magnitude of deviations between the two methods was not importantly related to the R.Q. obtained from the open-circuit measurement, or to the small differences of pulse rate observed in some cases. The average difference of pulse rate was 1 beat per minute greater during the open-circuit measurement than during the closed-circuit measurement. Familiarity of the pa-

TABLE 1. DIFFERENCES BETWEEN OPEN AND CLOSED-CIRCUIT MEASUREMENTS

	Cal./m.²/hr.		BMR (per cent)	
	All recordings	"Regular" recordings	All recordings	"Regular" recordings
Number	49	36	49	36
Mean difference	0.0	−0.1	0.0	−0.3
S.D. ±	2.60	2.17	7.45	6.22
S.D. ± corrected*	2.44	1.95	—	—

* Corrected to allow for test-to-test variation having S.D. of ±0.96 cal./m.²/hr. (6).

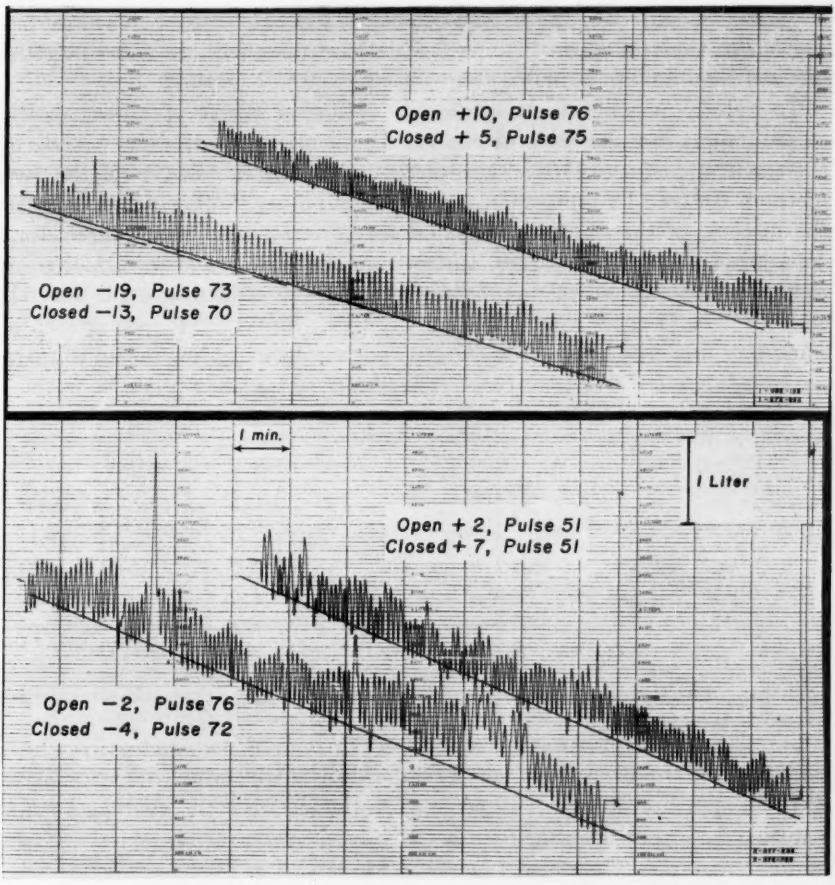

FIG. 2. Regular and irregular closed-circuit recordings of basal metabolic rate. Upper and lower portions each illustrate closed-circuit recordings of two persons. In the upper two recordings, there is more regularity of tidal volume and closer adherence of end-expiratory points to a linear slope than in the lower two recordings. Inspiration is upward. Values of pulse rate and BMR are given for these recordings and also for open-circuit measurements. (See text.)

tient with measurement of BMR did not appear to be related to the magnitude of deviations, since these were no smaller in 17 patients who previously had one or more tests in this laboratory. Similarly, 5 of the 13 "less regular" recordings were obtained on these 17 patients.

Additional data on the relative reproducibility of the two methods were obtained by comparison on 1 patient on different days, and by duplicate closed-circuit measurements on the same day on 2 other patients who were

having duplicate open-circuit measurements on each of three mornings per week for other purposes (Table 2). In these instances the closed-circuit method was less reproducible than the open-circuit method.

To determine whether the different values of BMR obtained by the closed-circuit method were of clinical significance, a review was made of the medical history of the 21 patients for whom the BMR by the two methods differed by more than 5 percentage points. Following a review of the history, of the results of physical examination and of the laboratory data other than BMR values, the two unidentified values of BMR were judged as to their clinical significance by one of us (Blackburn). It was felt that in 12 of 21 cases the clinical management would have been altered in some way had the closed-circuit value been accepted rather than the open-

TABLE 2. VALUES OF BASAL METABOLIC RATE

Patient		Closed circuit	Open circuit
1	Day 1	−12	−17
	Day 2	−28	−21
2	Test 1	− 8	−11.0
	Test 2	−12	± 1.0*
3	Test 1	− 5	− 9.5
	Test 2	−20	± 1.8*

* Mean ±S.D. of six tests performed within several days before and after closed-circuit measurements.

circuit value. In 10 of the 12 instances, this occurred when the two values were on opposite sides of a 15 per cent deviation from the average normal value.

COMMENT

Similar discrepancies between the results of consecutive measurements by open and closed-circuit methods have been reported by others. In 5 of 19 comparisons on 9 patients, Krogh and Rasmussen (7) found the closed-circuit values to differ by 6 to 11 per cent of the open-circuit value. Hunt (8) reported closed-circuit values within 12 per cent of the open-circuit values in 20 of 25 patients. A difference between methods of 5 per cent or more was found for 13 of 25 adults studied by Lewis and associates (4), and 10 per cent or more in 8 of 18 patients reported by Willard and Wolf (9). Also the standard error of closed-circuit measurement (2.3 cal./m.^{2}/hr.) derived from analysis of variance of multiple determinations by Harmon (10) was similar to the standard deviation of our comparisons.

A standard error of the closed-circuit method of about 2.3 cal./m.^{2}/hr., or about 6 to 7 per cent is of clinical importance, since this is almost equal

to the inter-individual variability of the healthy population, this standard deviation being about 2.5 cal./m.^{2}/hr. (6). Such a method is limited in its ability to detect other than large deviations from normality and to measure accurately changes of moderate magnitude.

The closed-circuit method is in almost universal clinical usage. In view of the limitations of available discussions, the basis of its errors merits consideration.

The correct determination of the "basal" metabolic rate requires first that the patient be in a "basal" condition, for either open-circuit or closed-circuit methods. A second requirement is that the rate of oxygen consumption be measured correctly.

The accuracy of the open-circuit method requires little justification, and as used here, the error has been estimated in the average case to be within 0.2 cal./m.^{2}/hr., or equivalent to less than 1 per cent of the usual metabolic rates (6). The conversion of oxygen consumption to caloric value may be in error by several per cent if the respiratory quotient of the expired air, which is used to establish the caloric value of oxygen, does not equal the respiratory quotient of metabolic processes. Such inequalities may result mainly from temporary overventilation or underventilation during the test; errors of less than 1 per cent result from neglecting the metabolism of protein, as is also done with closed-circuit methods (11). The relative susceptibility of the open-circuit method to other errors is noted later in this paper.

To assess the possible errors of the closed-circuit method, it is helpful to review the basis on which it was originally proposed by Krogh (12) and by Benedict (13). The subject rebreathes from a spirometer that contains an absorber for carbon dioxide. It initially contains oxygen at a partial pressure considerably greater than that of atmospheric air, to provide sufficient oxygen for the metabolic requirements and to prevent hypoxemia during rebreathing. The duration of the rebreathing period is usually six to ten minutes, because many persons become restless if it is further prolonged, and because of the construction of some types of apparatus. One measures an average rate at which the volume of the spirometer decreases. For this to equal the rate of metabolic utilization of oxygen, numerous conditions must be satisfied. Proper control of some characteristics of the apparatus, such as volumetric calibration, kymographic accuracy, temperature, completeness of carbon dioxide absorption and degree of aqueous saturation of spirometer gases, is often adequately provided by the manufacturer and his instructions. However, there are more difficult problems having to do with the patient.

Leakage of gases may occur around the mask, mouthpiece or noseclip. With the closed-circuit method, net inspiratory leaks add gas to the system

and cause falsely low values for oxygen consumption; tests are not usually made for such leaks. With the open-circuit collection of expired air, inspiratory leaks produce no error, if the composition of the air entering at the leak and at the inspiratory valve is the same. Expiratory leaks are more frequent. With the closed-circuit method, both inspiratory and expiratory leaks produce errors of equal volume to the leak, and the percentage error is the ratio of the leak rate to the metabolic rate. In contrast, outward leaks of expired air with the open-circuit method cause falsely low results, but the percentage error is the ratio of the leak rate to the total ventilation rate. Since the ventilation rate is about 25 times the oxygen consumption rate, a given expiratory leak in the open-circuit method causes only 1/25 the error it would produce in the closed-circuit method. In the latter, expiratory leakage of only 1 ml. per breath, or about 15 ml. per minute, produces a false elevation of BMR of 5 per cent or more. Although the usual method of noting a change of slope of the recording when a weight is placed on the spirometer bell will detect large leaks, it is doubtful if a change of slope of the order of 5 to 10 per cent can be detected accurately within the usually recommended period of one to two minutes, especially if the breathing is irregular and if detection must be made by visual inspection during the course of the procedure. However, such testing is imperative, although not always so acknowledged (14) or even possible on some commercially available apparatus. Not infrequently we have detected and corrected significant leaks, even though the patient indicated that nasal closure was complete as judged by his attempts to breathe with a closed mouth.

With the closed-circuit method, as noted by Krogh and by Benedict, the volume of the lungs, customarily end-expiratory, must remain constant or be the same at the beginning and end of the measured period. Otherwise the change of spirometer volume represents not only the decrease from oxygen utilization but also any change of lung volume. Although some authors have believed that progressive changes of lung volume could at times be detected by irregularities of the recording (15, 16), substantiating evidence was not presented, and this was shown not to be the case in 1 patient (9).

Errors resulting from changes of lung volume are proportional to the ratio of the change of lung volume to the total oxygen uptake during the period when the change occurs. With the open-circuit method the error ratio of the change of lung volume to the total ventilation is only 1/25 as large.

The closed-circuit system of lungs and spirometer contains gases that are exposed via the blood to the tissues. At the beginning of the rebreathing procedure, the partial pressure of alveolar oxygen increases and that

of alveolar nitrogen decreases. This causes an initial uptake of oxygen that raises its concentration in the blood but does not represent tissue utilization. Also nitrogen is released from the blood and tissues into the lungs. Unless the rates of this oxygen uptake and of nitrogen elimination are equal and opposite, or are zero, the rate of change of volume of the spirometer will not equal the rate of oxygen utilization. Krogh expressed the belief that sufficient equilibrium was attained within five minutes so that these effects were negligible thereafter. However, more recent studies on the rates of nitrogen elimination from lungs, blood and tissues following a change of inspired nitrogen tension have shown that equilibrium is far from complete in five minutes (17). It is difficult to estimate the quantitative extent to which this may affect the apparent rate of oxygen uptake, but alterations of the order of at least several per cent seem possible.

A major factor in evaluating the satisfactory nature of a closed-circuit recording has been its regularity, particularly of the end-expiratory level (15). Figure 2 (upper) illustrates two "regular" recordings, and two "less-regular" examples below. In the "regular" records, slopes of the last six-minute periods are unquestionably linear. They also differ significantly from the slope required to equal the open-circuit value, as is indicated by the broken line under the lower of the two "regular" recordings. The pulse rates give no indication that the metabolic levels were actually dissimilar and the R.Q. of 0.82 calculated from open-circuit collection of expired gases happened in both cases to be that assumed in the closed-circuit calculations. One possible explanation of the discrepancy in results could reside in the exchanges of nitrogen. A small, continued elimination of tissue nitrogen at a constant rate, combined with a constant oxygen utilization rate, could result in an apparent oxygen utilization rate that was linear but falsely low. The theoretical complexities arising from the varied rates of nitrogen exchange of different tissues, and from the decreasing volume of the spirometer, make it uncertain whether a period of nitrogen reabsorption could occur. If so, this could produce linear slopes but falsely high results.

Some irregular records agreed almost exactly with open-circuit results. First, irregularity of breathing pattern, as illustrated in the lower recordings of Figure 2, does not necessarily indicate the absence of an adequately relaxed or "basal" state. Secondly, the slope lines have been drawn on such records in a manner intended to minimize the effect of changes of end-expiratory lung volume. Errors resulting from such changes are proportional to the ratio of the change of lung volume to the total oxygen uptake during the period when the change occurs. Also, accuracy does not require that lung volume be constant throughout the test, but that it be the same at the beginning and end of the period of measurement. Accordingly, as

far as possible, one selects a slope connecting two points that are relatively widely separated in time, for example at five and ten minutes, and which represent the average end-expiratory volumes for two periods when the breathing pattern appears similar. In short, a regular linear recording very probably does not guarantee accuracy, nor is accuracy precluded by an irregular record.

Many authors have advocated the use of repeated tests on one or different days to minimize errors in estimating the BMR. Familiarity with the procedure undoubtedly is conducive to "basal" conditions with some persons. However, the acceptance of the lowest result of repeated tests as being the "most basal" vitiates the advantage gained in accuracy of the mean value of individual measurements that are each subject to random errors. In view of the reduction of possible errors of the closed-circuit method obtained by measurements covering the later part of a ten-minute recording, it is uncertain whether appreciable advantage results from repeated separate recordings of eight or less minutes' duration. Lewis and associates (4) found a correlation ($r=0.81$) between open-circuit values and the mean of two or more closed-circuit values that was no greater than our results ($r=0.84$) for single recordings. It should also be recognized that with a method having a standard error for individual measurements of 6 per cent, two consecutive results cannot be considered to differ significantly unless they vary by about 9 per cent, or more.

Repeated testing on different days has economic and other disadvantages, and one's confidence in its general utility is not augmented by Harmon's finding (10) that the correlation between values obtained by the closed-circuit method on the second and third days ($r=0.56$) of repeated tests was less than that between the results of tests on the first and second days ($r=0.70$).

It has been asked whether our experience with the closed-circuit method is sufficient to provide an adequate comparative test with the open-circuit method. This may be considered on the basis of adequacy of technic of measurement and of adequacy of the group studied.

This study was preceded by similar comparative measurements on 34 other patients. During the preliminary study a systematic difference of several per cent between methods became apparent early but was later eliminated after correction of small errors in both methods. In the series reported here, the precautions taken to ensure proper operation of the closed-circuit apparatus, as are noted earlier in the section on methods, very probably exceeded those employed in common clinical usage. If a learning factor were present in our application of the closed-circuit method it might be expected that the deviations between the two methods would become smaller with continued use. However, no significant difference was

observed between the variance of deviations of the first 25 and the last 24 comparisons.

The series of 52 comparisons is considered adequate in the following respects. During the series a concurrent analysis showed the frequency and magnitude of the differences between methods to be assuming an approximately normal distribution. About 50 such observations are sufficient to provide statistical assurance that the standard deviation of differences, noted in Table 1 to be 6 to 7 BMR percentage points, is unlikely to be less than about 5 percentage points, even if many hundreds of observations were obtained. That the group of 52 patients was representative of patients undergoing routine tests was shown by the correspondence of this group, in respect to distribution of age, sex and frequency of various levels of BMR, to another group of 391 patients tested during the second weeks of July and December, 1955.

SUMMARY

The basal metabolic rate was measured consecutively by open-circuit and closed-circuit methods on 52 patients undergoing routine tests. With the open-circuit method, satisfactory tests were obtained in all. With the closed-circuit method, nasal expiratory leaks that could not be eliminated within ten to twelve minutes occurred with 3 patients. The mean values for the remaining group by both methods did not differ significantly, but the standard deviation of the differences between methods, expressed as BMR, was about 7 per cent. In 21 of 48 cases, the BMR values differed by more than 5 per cent. It was felt that for 12 of the 21 patients, the clinical management would have been altered in some way had the closed-circuit value been accepted rather than the open-circuit value. For measurement of the rate of metabolic utilization of oxygen over periods of five to ten minutes, the closed-circuit method is liable to much greater errors than the open-circuit method. Reasons for this are presented.

Acknowledgment

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Editorial

IN DEFENSE OF THE BASAL METABOLIC RATE

ELSEWHERE in this *Journal* Fowler and his associates analyze the technical limitations of the closed-circuit method for the measurement of basal metabolism. It will come as no surprise to most physicians to learn that there is a considerable and irreducible variability in the basal metabolic rate as ordinarily determined for routine purposes. It has generally been assumed, however, that the inaccuracies observed with this test were chiefly associated with the basal postabsorptive state. There can be no question that failure to achieve the complete physical and psychologic relaxation demanded for the basal postabsorptive state, as defined, has always been a major pitfall and a very important source of error. Nevertheless, brief consideration of the accurate and reproducible estimations of basal metabolism possible with the open-circuit method as described by Fowler (and many years ago by Boothby and others) makes it clear that difficulties with the patient in achieving bodily and physical relaxation have been overemphasized as a source of error. The open-circuit method is just as liable to such handicaps as is the closed-circuit method, and yet with limited training of the patient the open-circuit method permits extraordinarily reproducible data to be obtained.

Fowler's observations provide ammunition for those who consider the inherent variability of the routine basal metabolic rate to be so great as to limit seriously its clinical usefulness. The closed-circuit method which he criticizes is the only one available to the great majority of physicians. Since the errors inherent in this technic cannot be eliminated with apparatus currently available, troublesome inaccuracies cannot be entirely avoided, no matter how much care is taken in the training of technicians or the conditioning of patients. Nevertheless, the proposition that the basal metabolic rate has now outlived its clinical usefulness and ought to be completely replaced by newer procedures (as has been suggested by a number of observers in recent years) deserves to be challenged. Such a view overlooks the inherent differences in the kind of information about the thyroid gland provided by the various types of clinical tests of thyroid function currently available to the clinician as well as the inherent advantages of the basal metabolic rate.

Chief among clinical tests advocated in place of the basal metabolic rate

are various radioiodine tracer tests and the determination of the serum level of protein-bound iodine. Clinical procedures involving the radioiodine tracer technic measure one of two aspects of thyroid function. Some measure, by one means or another, the rate at which radioiodine is accumulated and incorporated in thyroid hormone within the thyroid gland. Others, particularly estimation of the serum level of radioactive protein-bound iodine or conversion ratio, provide an index of the rate of hormonal secretion from the thyroid gland. Careful analyses of various radioiodine tests leave no reasonable doubt that the results of these tests can be extraordinarily specific in the diagnosis of exophthalmic goiter, particularly in mild form, and that they can be of considerable but less striking value in the diagnosis of several other types of thyroid disorder. However, results of radioiodine tests may mislead the unwary clinician as to the state of thyroid function in a number of circumstances. For example, in some patients who have previously undergone operations on the thyroid gland, or radiation therapy, or who have received antithyroid drugs, and in patients who have large endemic nodular goiters or lymphocytic thyroiditis, an abnormally high uptake of radioiodine may be interpreted erroneously as indicating hyperthyroidism, unless ancillary data also are carefully considered. Any circumstance which provides excessive concentrations of organic or inorganic iodine in the blood from exogenous sources may retard or abolish the uptake of iodine, regardless of the state of thyroid function.

The determination of the serum concentration of protein-bound iodine generally provides an accurate measure of the level of circulating thyroid hormone and, therefore, is usually a reliable guide to the over-all status of thyroid function. However, this procedure also has its pitfalls and limitations. Contrary to statements sometimes made about it, the protein-bound iodine technic has not proved practicable for small laboratories to perform accurately, and it remains at this writing a procedure which is technically difficult and expensive. As is the case with tracer tests, artefacts frequently may obscure values for protein-bound iodine in the plasma, these artefacts arising from the numerous sources of iodine, particularly organic iodine introduced into the blood stream in the diet, in medication and in diagnostic radiology. In most clinical practices the number of occasions on which the results of both radioiodine and protein-bound iodine tests may be rendered invalid by such artefacts is not small.

Apart from the disadvantages of its competitors, however, the basal metabolic rate possesses certain positive inherent advantages, not shared by protein-bound iodine, radioiodine tracer tests or other clinical adjuncts. The basal metabolic rate may be said to reflect primarily the impact of the thyroid hormone upon the organism. Aside from the question of diagnosis, the quantitative evaluation of the severity of a thyroid disorder be-

comes of prime importance to the physician concerned with caring for a patient. In this respect, as pointed out by Frasier, the basal metabolic rate may, despite its faults, frequently prove superior to the other procedures available. The basal metabolic rate usually but not always appears to parallel with considerable reliability the extent of clinical hypothyroidism or hyperthyroidism present. The content of protein-bound iodine in the plasma also shows such quantitative correlation, but perhaps less decisively. On the contrary, results of radioactive iodine tracer tests show very little parallelism with the condition actually present. Furthermore, the basal metabolic rate often provides an unexcelled means of following the progressive changes in a patient in response to treatment, whereas all too often treatment makes it impossible to estimate the content of protein-bound iodine in the plasma and the uptake of radioiodine. The basal metabolic rate remains particularly useful as the guide to replacement therapy in hypothyroid states.

The basal metabolic rate also provides useful information in several clinical disorders in which thyroid function is entirely normal. This circumstance sometimes has been adduced as an argument against its use. However, more than once the first clue to the existence of a pheochromocytoma or an unrecognized blood dyscrasia has been an inexplicable elevation in the basal metabolic rate in a patient whose clinical examination failed to disclose other evidence of hyperthyroidism. Elevated basal metabolic rates also provide some explanation of, and insight into, some of the disturbing symptoms which accompany severe essential hypertension, such as sweating, intolerance to heat and loss of weight.

Perhaps the most cogent argument in favor of retaining the basal metabolic rate, despite its shortcomings, in routine clinical practice at present is its augmented usefulness when it is used in conjunction with alternative and newer procedures. A patient with a difficult thyroid problem whose basal metabolic rate has been determined carefully and accurately and who also has had protein-bound iodine and radioiodine tracer tests is one in whom it is possible to evaluate the thyroid function with a degree of precision not considered possible a few years ago. Since each of these procedures provides a kind of information different from the others, all supplement each other in a very useful way.

For the foregoing reasons the current trend away from use of the basal metabolic rate should be decried. Perhaps some means will be found to circumvent the technical difficulties which limit the great potential usefulness of this fundamental clinical measurement. It seems improbable that existing closed-circuit apparatuses can be modified so as to eliminate the shortcomings enumerated by Fowler and his associates. Perhaps recognition of this situation will induce some enterprising manufacturer to develop a

practical, modern instrument that will permit the average hospital or physician to measure the basal metabolic rate by means of the more accurate open-circuit method.

The basal metabolic rate was one of the first fundamental parameters to be extensively investigated in clinical medical practice. It has a long and honorable record of clinical usefulness, and seems likely to continue to be a valuable clinical adjunct. Suggestions that it is ready to be discarded seem premature and ill considered, despite the limitations which have been described.

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Letters to the Editor

HAS ENDEMIC CRETINISM ANY RELATION TO THYROID DEFICIENCY?

To the Editor:

The statement of Paracelsus and Malacarne that goiter and endemic cretinism have a common origin, and the affirmation of Stanbury and Querido (*J. Clin. Endocrinol. & Metab.* **16:** 1522 (Nov.)1956) that this cause is iodine deficiency do not seem to many observers to be definitively proved, especially in our country.

The Piedmontese Commission nominated by the King of Sardinia, from whose report has been taken the picture of a goitrous endemic cretin (not from Sardinia, but a 23-year-old man from Valpelline, in the Aosta valley), stated in 1848 that goiter is merely an accidental accompaniment of cretinism and that goiter and cretinism differ essentially in their pathologic nature. In that classic report we find the pictures of 9 cretins, each with a short comment. If we glance at the figure reproduced in Stanbury and Querido's article (this *Journal*, Nov. 1956) we do not see in any way the "short, thick, curved-in, little fingers" (Means) of juvenile myxedema. Three other cretins were hydrocephalic, since their antero-posterior cranial diameter was 34–36 cm., and the transverse diameter 30–34 cm. Two of these 9 cretins were brothers; their parents were normal and in good health, and bred 6 sons. Although they lived always in the same place (Valpelline) and under the same conditions, the first 3 were cretins but the second 3 were normal and very intelligent. These and similar observations led the Commission to formulate the opinion, also held by us, that endemic cretinism is a typical *constitutional* disease.

A cretin with hyperthyroidism has been described by Hurxthal, and another by myself (1). Stanbury and Querido judge that such cases are not surprising in view of the histologic picture of the gland; among the widespread cellular degeneration and fibrosis, some hypertrophic islands might prove capable of sufficient hyperplasia to produce thyrotoxicosis. Perhaps this is so, but it is really surprising that among 50 patients all fitting the common textbook description of cretinism that Stanbury has examined in Mendoza, Argentina, not one could be recognized "from skin temperature and texture, and from the pulse" as an hypothyroid patient. What is more,

"most of these subjects had a normal concentration of protein-bound iodine, but 2 had abnormally high values." How is it conceivable that, after having suffered at an early age "the most severe inroads which the morbific influences causing the endemic are capable of producing" (Means (2)) *all* those thyroids managed to achieve a normal function?

Since 1952 we have studied the metabolic pattern of the endemic cretin. Among the 21 reported by Vogliazzo and other coworkers of ours, none showed a low B.M.R. or a high level of plasma cholesterol (except 2 very old patients). The values for iodine metabolism were not precisely in the hypothyroid zone, but their significance was not clear because of iodine prophylaxis in that valley. In other groups of cretins studied where iodine prophylaxis had not been carried out, we found a B.M.R. either normal or, more often, above normal; a normal or subnormal level of blood cholesterol; and an average serum protein-bound iodine level of 4.45 gamma per 100 ml. (doubled after treatment with TSH). The thyroidal radioiodine uptake was prompt and high, the average being 49.2 per cent at the first hour, and 49.05 per cent at the twenty-fourth hour (in our experience, normal uptakes are respectively 10.59 and 30.11 per cent). We found similar values in young people with endemic goiter who were in good physical and mental condition. We wonder if the abnormal findings with regard to iodine metabolism in the goitrous thyroid, so exhaustively investigated in the excellent studies of Stanbury *et al.*, prove that endemic goiter is a consequence of lack of iodine in the place where these patients were living. We found similar high uptakes in school children living in areas of endemic goiter, whose thyroids were clearly hyperplastic, though the iodine content of the drinking water and their daily urinary excretion of iodine were in the normal range. Furthermore, if a school-child living in an area where there is no endemic goiter and where the parameters of thyroidal iodine metabolism in his school-fellows are normal, has thyroid hyperplasia, the values for his own iodine exchange are similar to those of a boy with endemic goiter. Could not these manifestations of abnormal iodine metabolism in the thyroid be the consequence of, instead of the origin of, the goiter? In the same way, the incretion of abnormal iodinated substances may be the cause of the goiter, because of the low metabolic and calorigenic capacities of these substances (as in the cases of goitrous cretinism caused by inborn errors of metabolism, reported by Stanbury). However, this incretion may sometimes be the consequence of some pathologic process that has affected the thyroid; for example we have found diiodotyrosine in the blood during the swelling of the thyroid in acute thyroiditis.

In conclusion, we believe that endemic cretinism is an extremely complex illness, in which thyroidal function may be impaired, though usually not to the extent of serious failure. Even then, endemic cretinism is quite dif-

ferent in its pathogenesis from Gull's disease (myxedema). We suggest that the alterations in iodine metabolism that have been described in endemic goiter, are usually the consequence of, rather than the cause of, the goitrous process.

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ON THE NATURE OF ENDEMIC CRETINISM

To the Editor:

We are grateful for the opportunity to reply to the letter of Professor Costa and to thank him for calling attention to our error in attributing the cretin of Figure 1 in our recent paper to Sardinia, when in fact, the patient lived in northern Italy. The original of this classic monograph was not available to us. The photograph was provided by Dr. William Merrington from a copy extant in the British Museum. The cause of cretinism in the unfortunate subject of the photograph will perhaps never be known, but evidently he was recognized at the time as a patient with endemic cretinism.

Professor Costa has rightly raised interesting questions regarding the nature of endemic cretinism. This problem has been debated for years. Perhaps the most compelling argument in favor of a deficiency of iodine as the causative factor in endemic cretinism is the remarkable disappearance of the disease when adequate methods of iodine prophylaxis are introduced. The contrary evidence, namely, that endemic cretins have been shown to have normal blood concentrations of protein-bound iodine and normal or high thyroidal retentions of radioactive iodine, has been cited to indicate that iodine deficiency has nothing to do with the disease. It should be pointed out, however, that measurements of iodide metabolism in an adult endemic cretin have nothing to contribute regarding the state of iodine metabolism at the time when the irreversible structural damage was done. Indeed, the modern-day finding of normal thyroidal function in these patients might be expected, in view of the widespread use of iodide prophylaxis and the improved nutrition in the countries where such studies have been made. One wonders what the blood concentration of hormonal iodine might be in some of the rare remaining areas of the world where

severe endemic goiter exists, and where endemic cretinism is commonplace.

One is at a loss to understand the nature of the thyroid disorder which leads to the abnormal values for iodine metabolism seen in Dr. Costa's patients with endemic goiter. Already at the end of one hour, half the administered dose was in the gland, and similarly at twenty-four hours. This could only be so if intermediate values were much higher. The finding is consistent either with an extreme degree of iodine depletion or some sort of metabolic derangement of the thyroid. If indeed these patients were ingesting a normal amount of iodine, then it would be of the greatest interest to explore in more detail the character of the defect exhibited by these patients.

Our own studies with endemic goiter both in Argentina and in Holland merely indicated that the observed facts were consistent with a single operating factor, *viz*, a deficiency of iodine. Thus, there was a clear correlation between iodine deficiency and the avidity of the gland for labeled iodine. When iodide was administered, the function of the gland returned toward a normal state. It is impossible to extrapolate from our studies to those of Professor Costa.

We cannot believe that the present facts warrant the exclusion of iodide deficiency as the sole cause of endemic cretinism. Professor Costa, however, has done a service in re-opening the possibility that endemic cretinism is a disease, *sui generis*, with only secondary manifestations in the thyroid. Admitting this for the moment, one wonders then how he can define endemic cretinism. What are its essential characteristics, and how does one proceed to make a diagnosis? Perhaps more pertinently, Professor Costa has indirectly re-opened the problem of whether constitutional, nutritional or environmental factors may, in conjunction with iodine deficiency, lead to cretinism. There seem to be good reasons to support such a point of view, but it is still necessary to clarify and define them. Tools are at hand now for performing this task. The fact remains that even though endemic cratinism has been completely defined in one locale, the situation may be entirely different in the next valley.

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SYNERGISM AND STRAIN DIFFERENCES IN THE ASSAY OF URINARY GONADOTROPINS

To the Editor:

A common standard has been suggested for the assay of human urinary gonadotropin (1, 2). Most methods of assay would yield a positive response with either FSH or ICSH or, synergistically, with a mixture of the two. The use of a common standard for such assays can only be justified if it and all the extracts assayed against it are composed of pure FSH or pure ICSH or of a constant mixture of the two. Some workers claim that there are no qualitative differences in human urinary gonadotropins (3). Others claim to show differences because different types of assays furnish conflicting results for comparison of the same materials (4, 5, 6). This suggests that if different strains of animals differ in relative sensitivity to FSH and ICSH, then assays by the same method but using different colonies of animals may also give conflicting results. This possibility has been tested, using the response of the uterus of intact immature mice. The possibility of synergism between the extracts used has also been investigated.

Four pairs of extracts which were thought to be qualitatively different were selected for testing. All were prepared by the same kaolin-acetone method. For 3 pairs, the urine was obtained from the same patients before and during treatment with cortisone or estrogen: for 1 pair, the urines were from different patients.

Immature albino mice were obtained from 2 dealers. Those from Dealer 1 were used in a quantal assay with doubling of uterine weight as the endpoint (7). Those from Dealer 2 were given injections in the same way but the results were calculated from the graded uterine response (8). The methods of calculation were those of Gaddum (9).

In tests of synergism, solutions were made from both extracts separately and the two were mixed in the proportions shown in Table 1. Equal volumes of the resulting solutions, labelled with code numbers to avoid psychologic bias, were injected into groups of 4 or 5 mice from Dealer 1. The timing of injections and selection of animals were the same as in the assays previously mentioned, and the mice were killed after the same interval. The results in Table 1 show that in Pairs 2, 3 and 4 there was evidence of synergism between the two extracts, the response of the uterus to one of the mixtures being significantly greater than to either solution alone. In each experiment the response of the ovaries was of same pattern as that of the uterus, and in Pair 4 the response showed significant evidence of syner-

TABLE 1. UTERINE WEIGHT OF MICE TREATED WITH PAIRS OF GONADOTROPIC EXTRACTS MIXED IN VARIOUS PROPORTIONS

Proportion of the two extracts mixed	Uterine weight (mg.) (mean ±S.E.)					
	5:0	4:1	3:2	2:3	1:4	0:5
Pair						
1	8.6 ± .95	6.8 ± .63	13.6 ±5.21	8.9 ±2.75	18.3 ±3.58	12.5 ±1.66
2	15.6 ±2.95	38.6* ±5.64	7.8 ±2.00	8.5 ±1.35	6.3 ± .16	7.1 ± .82
3	6.5 ± .27	5.4 ± .27	8.1* ± .59	6.8 ± .72	6.2 ± .27	6.3 ± .18
4	5.5 ± .46	9.3 ±2.10	4.8 ± .17	4.5 ± .30	33.4* ± .73	6.2 ± .34

* Significantly different ($P < .05$) from means for either extract alone, *i.e.*, 5:0 and 0:5 "mixtures."

TABLE 2. RELATIVE POTENCY OF PAIRS OF GONADOTROPIC EXTRACTS ASSAYED IN TWO STRAINS OF MICE

Source of mice	Dealer 1			Dealer 2		
Pair	Relative potency (%)	λ	P* (testing parallelism)	Relative potency (%)	λ	P* (testing parallelism)
1	193 (146–235)	0.10	0.7–0.8	135 (116–183)	0.11	0.8–0.9
2	44 (36–56)	0.07	0.2–0.3	85 (69–100)	0.08	0.3–0.5
3	92 (70–136)	0.16	0.2–0.3	42 (23–65)	0.13	0.3–0.5
4	89 (57–155)	0.22	0.9	108 (64–204)	0.20	0.2–0.3

* A value <.05 would suggest a significant departure from parallelism.

gism ($P<.05$). This synergism indicates qualitative differences between the two extracts and it is remarkable how sharply the optimum mixture is defined. This explains why a mixture of equal parts of two extracts may fail to show synergism, though the extracts differ.

The relative potency of the two extracts for each pair was assayed, using both strains of mice: thus there were 8 assays. In Pairs 1 and 2, the assays using the different strains were performed at different times, but in Pairs 4 and 5 they were performed simultaneously and all the mice were given injections from the same solutions of the extracts. Table 2 lists the results, with fiducial limits ($P=0.95$) and the other statistical data. The values for P show no departure from parallelism and there was no departure from linearity in 3 of the assays which were of more than four-point design. The assays were therefore satisfactory, and it is seen that in 2 cases significantly different results were obtained for comparison of the same extracts when different strains of mice were used. In each case the mice from Dealer 1 appeared relatively more sensitive to the extract thought to contain more ICSH.

These results are further evidence that there are qualitative differences in human urinary gonadotropins, and that similar assays in different strains of animals may yield different results. The extracts were specially selected to demonstrate these effects which might not otherwise have been apparent, but if different workers propose to use a common standard to compare their results the possibility of such effects must be taken into account.

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Acknowledgments

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The Endocrine Society

THE 1957 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada. Dr. Carl R. Moore was the recipient in 1955 and Dr. Frederick L. Hisaw in 1956.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology.

Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

NINTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY AND THE MEDICAL COLLEGE OF GEORGIA

Augusta, Georgia, October 21-25, 1957

The faculty will consist of 24 eminent clinicians and investigators from various parts of the country in the fields of endocrinology and metabolism. The program will cover the various endocrinopathies, with emphasis on the clinical aspects, demonstration of laboratory tests, presentations of cases, and question-and-answer panel discussions. The course is designed to cover the main aspects of diagnosis and therapy in the field of endocrinology and metabolism for the physician in general practice and for those in other specialities who wish to have a general knowledge of this rapidly growing field.

A syllabus with brief abstracts of lectures will be available to the registrants at the time of the Assembly.

For further information concerning the program and registration, write to *Dr. Robert B. Greenblatt, Department of Endocrinology, Medical College of Georgia, Augusta, Georgia.* Registration is limited to 100; the tuition fee is $100.00. Rooms will be reserved for the students and faculty at the Bon Air Hotel. Residents and fellows will be admitted for $35.00.

FACULTY

A. Albert, M.D., Professor of Physiology, Mayo Foundation; Head, Endocrinology Laboratory, Mayo Clinic.

William E. Barfield, M.D., Associate Clinical Professor of Endocrinology, Medical College of Georgia.

Frederic C. Bartter, M.D., Chief, Section of Clinical Endocrinology, Clinic of General Medicine, National Heart Institute, National Institutes of Health.

Alfred M. Bongiovanni, M.D., Director, Endocrine Clinic, Children's Hospital of Philadelphia, Associate Professor of Pediatrics, University of Pennsylvania.

Thaddeus S. Danowski, M.D., Professor of Research Medicine, University of Pittsburgh School of Medicine.

Brown M. Dobyns, M.D., Ph.D., Associate Professor of Surgery, Western Reserve University, and Assistant Director of Surgery, Cleveland City Hospital.

Frank L. Engel, M.D., Associate Professor of Medicine, Assistant Professor of Physiology, Duke University School of Medicine; Director, Division of Endocrinology, Duke Hospital.

Roberto F. Escamilla, M.D., Clinical Professor of Medicine, University of California School of Medicine.

Thomas Findley, M.D., Professor of Medicine, in charge of Cardiovascular Research, Medical College of Georgia.

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Edwin C. Jungck, M.D., Assistant Clinical Professor of Endocrinology, Medical College of Georgia.

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Virgil P. S. Sydenstricker, M.D., Professor of Medicine, Medical College of Georgia.

Nathan B. Talbot, M.D., Associate Professor of Pediatrics, Harvard University; Physician, Children's Medical Service, Massachusetts General Hospital.

Henry H. Turner, M.D., Clinical Professor of Medicine, University of Oklahoma School of Medicine; Chief, Endocrine Clinic, University Hospitals.

Judson J. Van Wyk, M.D., Assistant Professor of Pediatrics, University of North Carolina School of Medicine; Assistant Attending Pediatrician, North Carolina Memorial Hospital.

PROGRAM

Monday, October 21, 1957

8:00– 9:00 A.M.	Registration: Place—Lobby of Talmadge Memorial Hospital, Medical College of Georgia
	Welcome by the President of the Medical College of Georgia
	Introduction to Endocrinology and the Endocrine Patient
9:00–10:00 A.M.	Introduction to Endocrinology—*Dr. C. N. H. Long*
10:00–10:10 A.M.	Intermission
10:10–11:15 A.M.	Evaluation of the Endocrine Patient.
	Medical—Dr. Escamilla
	Ob.-Gyn.—Dr. Greenblatt
	Pediatric—Dr. Van Wyk

11:15–12:15 P.M. Laboratory Procedures in Endocrinology—*Drs. A. Albert and A. Bongiovanni*

12:15–12:30 P.M. Questions and Answers

12:30– 2:00 P.M. Lunch

2:00– 5:30 P.M. Disorders of the Hypothalamus and Pituitary Body:
Panel Discussion with Case Presentations
Physiology and Chemistry of the Pituitary Body and Its Hormones, Hypothalamic Syndromes, Clinical Syndromes of Hypo- and Hyperfunction of the Adenohypophysis, Surgical Aspects of Pituitary Tumors, Medical Management of Hypo- and Hyperpituitarism, Therapeutic Hypophysectomy—*Drs. R. Escamilla (Moderator), A. Albert, C. Lloyd, C. N. H. Long and G. Smith*

7:00 P.M. Get-Acquainted Cocktail Party—Bon Air Hotel.

Tuesday, October 22

9:00 A.M.–12:30 P.M. Thyroid Function and Disease:
Panel Discussion with Case Presentations
1. Physiology of the Thyroid, Thyroid Function Tests, Hyperthyroidism, Hypothyroidism—*Drs. R. Rawson (Moderator), A. Albert, R. Keating, H. Turner and J. Van Wyk*

12:30– 2:00 P.M. Lunch

2:00– 5:30 P.M. Thyroid Function and Disease:
Panel Discussion with Case Presentations
2. Thyroid Adenoma, Colloid Goiter, Thyroid Cancer, Thyroiditis, Malignant Exophthalmos, Use and Abuse of Thyroid Hormone—*Drs. B. Dobyns (Moderator), R. Keating, R. Rawson and J. Van Wyk*

Wednesday, October 23

9:00 A.M.–12:30 P.M. Disorders of the Adrenal Cortex:
Panel Discussion with Case Presentations
Physiology and Chemistry of the Adrenal Cortex, Adrenal Function Tests, Spontaneous and Induced Adrenal Insufficiency Syndromes, Hyperadrenalcorticism, Adrenal Steroid Therapy —*Drs. F. Bartter (Moderator), A. Bongiovanni, F. Engel, W. Parson and V. P. Sydenstricker*

12:30– 2:00 P.M. Lunch

2:00– 5:30 P.M. Problems in Pediatric Endocrinology:
Panel Discussion with Case Presentations
Endocrine Disturbances in Growth and Development, Ambisexual Development, Hermaphroditism, Sexual Precocity—*Drs. N. Talbot (Moderator), A. Bongiovanni, M. Grumbach and J. Van Wyk*

Thursday, October 24

9:00 A.M.–12:30 P.M. Problems in Female Endocrinology:
Panel Discussion with Case Presenations
Physiology of the Ovary, Laboratory Evaluation of Ovarian Function, Ovarian Hypofunction Syndromes including the Menopause, Ovarian Dysfunction Syndromes, Use and Abuse of Sex Hormone Therapy in the Female, Infertility—*Drs. R. Greenblatt (Moderator), W. Barfield, E. C. Hamblen, and C. Lloyd*

12:30– 2:00 P.M. Lunch

2:00– 3:45 P.M. Disorders of Gonadal Function in the Male:
Panel Discussion with Case Presentations
Physiology of the Testis, Evaluation of Male Gonadal Function, Syndromes of Hypogonadism, Use of Male Sex Hormone—*Drs. E. C. Hamblen (Moderator), M. Grumbach, E. Jungck and H. Turner*

3:45– 4:00 P.M. Intermission.

4:00– 5:00 P.M. *Lecture: Hormones and Cancer—Dr. R. Rawson*, introduced by Dr. E. R. Pund, President of the Medical College of Georgia

5:00– 5:30 P.M. Questions and Answers.

8:00 P.M. Buffet Supper at the Old Medical College, for registrants, visiting faculty and the faculty of the Medical College, through the courtesy of the Medical College of Georgia Foundation

Friday, October 25

9:00 A.M.–12:30 P.M. Problems in Electrolyte and Water Balance:
Panel Discussion
Sodium, Potassium and Water Homeostasis, Primary and Secondary Aldosteronism, Diabetes Insipidus—*Drs. D. Seldin (Moderator), F. Bartter, T. Danowski, N. Talbot and T. Findley*

12:30– 2:00 P.M. Lunch

2:00– 4:30 P.M. Disorders of Calcium and Bone Metabolism:
Panel Discussion with Case Presentations
Function of Parathyroids, Hyper- and Hypoparathyroidism, Osteoporosis, Osteomalacia—*Drs. F. Engel (Moderator), F. Bartter, R. Keating and W. Parson*

4:45– 5:30 P.M. General Question-and-Answer Period

TRAINING PROGRAM FOR STEROID BIOCHEMISTRY

Applications are now being accepted for the second course in the Training Program for Steroid Biochemistry to start October 1, 1957. The program is conducted through the cooperative effort of the Worcester Foundation for Experimental Biology, with the Department of Chemistry, Clark University, at Worcester, Massachusetts, and the Department of Biochemistry, College of Medicine, Univ. of Utah, at Salt Lake City, Utah.

The training program is sponsored by the National Cancer Institute of the National Institutes of Health, U.S.P.H., to provide specialized training for individuals interested in steroid investigation. Two groups of candidates will be selected and will receive stipends during the period of training.

Post-doctoral candidates having an M.D. or Ph.D. degree will receive $5000 for one year starting October 1, 1957. The training will consist of laboratory and lectures covering theoretical and practical aspects of steroid research, and an opportunity to engage in a research problem under an established investigator.

Candidates having a B.S., M.S. or equivalent degree will receive $1500 for a six months training period, October 1, 1957 through March, 1958. The pre-doctoral program is intended to provide competency in the analysis of steroid compounds for research and clinical laboratories.

The closing date for applications will be *August 15, 1957*. Requests for applications should be made to Dr. Kristen Eik-Nes, Department of Biochemistry, College of Medicine, University of Utah, Salt Lake City, Utah, or to Dr. Frank Ungar, Department of Chemistry, Clark University, Worcester, Massachusetts.

THIRD INTERNATIONAL SYMPOSIUM ON RADIOACTIVE ISOTOPES IN CLINICAL MEDICINE AND RESEARCH

This meeting will be held in Bad Gastein, Austria, *April 7–10, 1958*, and will be devoted in part to the use of isotopes in endocrinology. It is hoped that many Americans will take this opportunity to meet their European colleagues. Inquiries with regard to presentation of papers should be made to: Herbert Vetter, M.D., Radioisotopes Laboratory, 2nd Medical University Clinic, Vienna 9, Austria.

CORRECTION

In the article entitled "Thyroid Function Studies in Children: Normal Values for Thyroidal I^{131} Uptake and PBI^{131} Levels up to the Age of 18" by L. Oliner, R. M. Kohlenbrener, T. Fields and R. H. Kunstadter (*J. Clin. Endocrinol. & Metab.* **17:** 61–75 (Jan.) 1957) the authors wish to correct an inadvertent misquotation in Table 5. In the column headed "Counting equipment" the item referring to Van Middlesworth should read "Scint., 12.7 cm. dist. from neck" instead of "Scint., against neck."

BOOK REGISTER

Biochemical Individuality. The Basis for the Genetotrophic Concept. By ROGER J. WILLIAMS, Professor of Chemistry and Director, Biochemical Institute, University of Texas; President of the American Chemical Society for 1957. Foreword by ALAN GREGG. This is the first work to collect the available information on individual differences in biochemical make-up, and to show how some critical problems may be solved through understanding of individuality. Hereditary factors are discussed. There is a chapter on endocrine activities. Medical scientists, biochemists, psychologists, anthropologists and educators will find this a valuable book; 214 pages; 17 figures; 1956. John Wiley & Sons, Inc., New York. Price $5.75.

La Puberté. Étude clinique et physiopathologique. By GUY LAROCHE, A. ASSAILLY, H. BRICAIRE, G. BROUET, E. FERRON, G. HUC, CL. LAROCHE, J. LEPRAT, P. MAURICE, H. SIMMONET, R. TOURNEUR and J. TRÉMOLIÈRES. This is an entirely new second edition. In recent years the clinical and biologic studies of puberty have progressed to the point where the whole subject needs a fresh review, as it has an important bearing on problems of medicine, biology, psychology, hygiene and education. Included are sections on the adrenocortical hormones, the hypothalamus, endemic goiter, tuberculosis, nutrition, and mental and gonadal disorders; 394 pages; 42 figures; 1956. Masson et Cie, Editeurs, Paris. Price 2,800 fr.

Rythmes et cycles biologiques. By ALAIN REINBERG and JEAN GHATA. A discussion of the elementary rhythmic activities of living matter—plants, insects, mammals and man; 128 pages; 9 figures; 1957. Presses Universitaires de France, Paris.

The Evaluation of Therapeutic Agents, with Special Reference to the Tranquilizing Drugs. By STEWART WOLF, M.D., Professor and Head, Department of Medicine, University of Oklahoma School of Medicine, Oklahoma City. Presented Oct. 10, 1956 as the Academic Lecture at the Eighth Mental Hospital Institute held in Denver, Colorado. Monograph Series No. 2, Mental Hospital Service, January 1957; 23 pages; 14 figures. American Psychiatric Association Mental Hospital Service, 1785 Massachusetts Ave., N.W., Washington D. C. Price $1.00.

The Metabolic Response to Neonatal Surgery. By P. P. RICKHAM, M.S., F.R.C.S., D.C.H., Consultant Paediatric Surgeon, Royal Liverpool Children's Hospital and Alder Hey Children's Hospital, Liverpool, England; Arris and Gale Lecturer, Royal College of Surgeons of England, 1956. This book presents the first careful investigation into the metabolic response to surgery of newborn infants. It describes detailed studies on 9 newborn infants undergoing various operations, and the findings are interpreted in the light of less complete studies on 300 newborn infants operated on for urgent conditions. The wide difference in the reactions to surgical trauma of the newborn as compared with adults and older children is discussed, and preoperative and postoperative regimens for infants are set forth; 93 pages; 9 tables; 9 charts; 1957. A Commonwealth Fund Book, Harvard University Press, Cambridge, Mass. Price $5.00.

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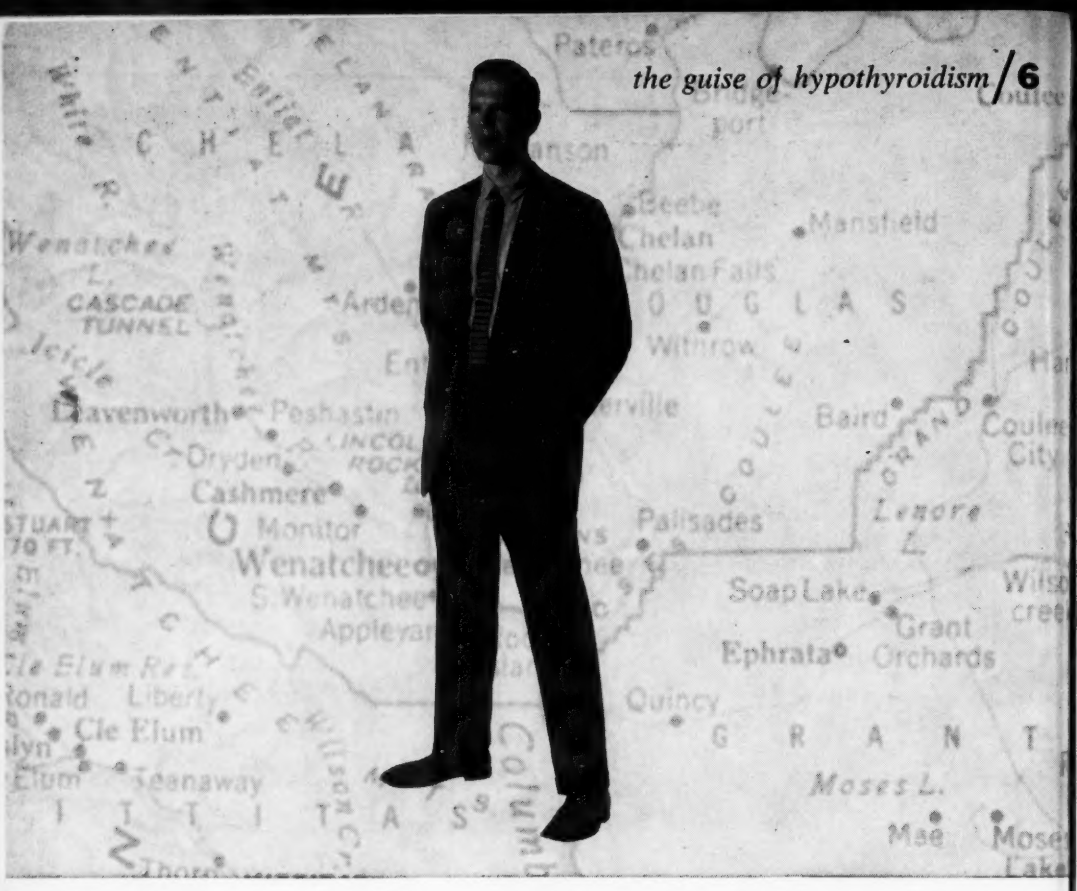

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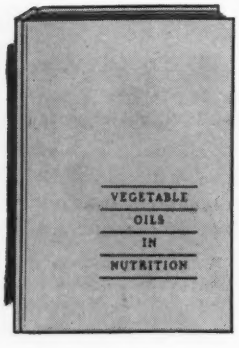

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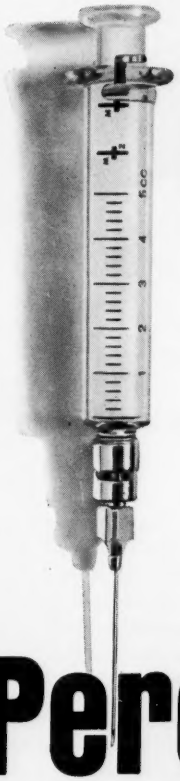

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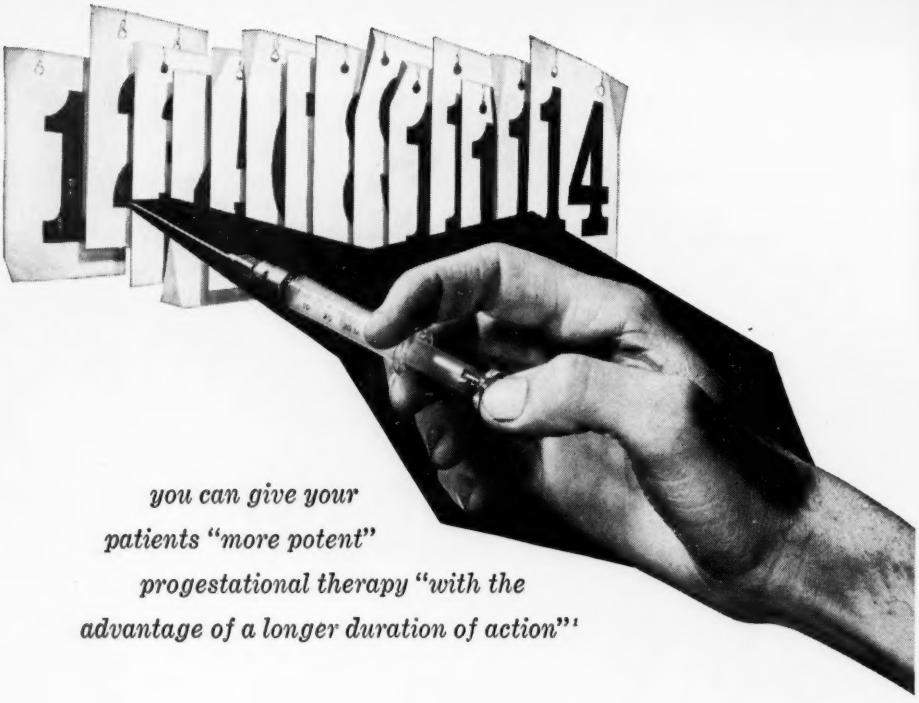

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